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A LABORATORY MANUAL
OF
ORGANIC CHEMISTRY
FOR
MEDICAL STUDENTS

BY

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PREFACE TO THE SECOND EDITION

THE book has been revised and enlarged in an attempt to bring it up to date. The chapters on Halogens, Alcohols, Amines, Amides and Cyanogen Compounds, Lipins and Carbohydrates have been expanded, and a new chapter on Colloids has been introduced.

The chapter on colloids was written at the request of the publishers. The dearth of elementary experiments on this topic is well known. The experiments were taken from unpublished notes by the author on "Physical Chemistry for Medical Students"—a course required of first-year medical students at the Long Island College Hospital.

The author wishes to express his indebtedness to Dr. Frank L. Haley for his aid in devising some of the new methods, especially the method on the preparation of levulose from dahlia tubers.

It is a pleasure to testify to the courtesy and consideration extended to the author by the publishers.

M. S.

NEW YORK, April, 1920.

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PREFACE TO THE FIRST EDITION

This manual was originally compiled as a laboratory guide in organic chemistry for the medical students of the Long Island College Hospital, Brooklyn, New York.

While writing these notes, the author kept one object constantly in view, namely, the selection of experiments that would be of real value to medical students.

The recent development of biological chemistry has created a demand for a much broader training in experimental organic chemistry than was formerly required of medical students. In this manual an attempt has been made to fulfill this need without burdening the student with a mass of unessential data.

In nearly every experiment definite quantities of chemicals have been specified, the object being twofold: to prevent the unnecessary waste of material, and to insure the success of the experiment.

Each student is expected to record his own observations, and for this reason alternate pages of the manual have been left blank.

To induce the student to correlate the facts observed in the laboratory with the theoretical matter taught in the lecture room and in the text-books, the experiments are accompanied with questions which the student is supposed to answer.

Much of the data contained in the chapter on alkaloids was obtained from Autenrieth's book "Detection of Poisons and Powerful Drugs." Authorized translation by W. H. Warren, Ph.D. (Blakiston). The author wishes to express his gratitude to Messrs. Blakiston &

Company for the courtesy shown in permitting the use of these data.

The author also wishes to acknowledge his indebtedness for many valuable suggestions obtained from the laboratory notes prepared for the use of medical students by Professor William J. Gies, of Columbia University, and from many standard texts on organic chemistry.

MATTHEW STEEL.

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PRACTICAL COURSE

IN

ORGANIC CHEMISTRY

CHAPTER I

QUALITATIVE ANALYSIS OF ORGANIC COMPOUNDS

1. The Detection of Carbon and Hydrogen. Dry a clean test-tube in a gas flame. Fit it with a cork through which passes a glass tube, bent at a right angle. Mix in a mortar a little dry cane sugar and ten times as much dry CuO; pour this mixture into the test-tube; cork; dip the outside end of the glass tube into baryta solution contained in another test-tube. Heat the sugar mixture over a flame.

Write answers to the following questions in your notebook:

a. What causes the drops of water which are condensed on the cool part of the glass test-tube and tube?

b. To what is the cloudiness which develops in the baryta solution due?

c. Write all equations involved.

2. Detection of Nitrogen in an Organic Substance. *a. As ammonia.* Grind a small portion of the substance under examination (e.g., about $\frac{1}{2}$ gram of casein) with about 2 grams of soda-lime. Place the mixture in a dry test-tube and heat. Note the odor of the fumes that are given off. If the odor of ammonia is not distinct, moisten a piece of red litmus paper in distilled water and hold it in the fumes. It should turn blue.

b. As sodium cyanide. (*Lassaigne's method.*) By means of a clamp fix a small, dry, clean test-tube in a vertical position and introduce into it a piece of freshly cut metallic sodium about the size of a pea. Gently heat the tube until the sodium has melted and begun to vaporize. Then add in minute portions the substance to be tested (e.g., dried egg-albumin). Continue the heating for about half a minute; cool the tube; break the lower end of it in a mortar containing 2 c.c. of alcohol; and, finally, add about 10 c.c. of water as soon as effervescence has ceased. (Care must be taken at this point, as small pieces of sodium are sometimes left unattacked by the alcohol and are liable to be thrown into one's face.)

Add 5 drops of dilute sodium hydroxide solution to the liquid in the mortar and filter. Place a portion of the filtrate, which should be clear and colorless, in a test-tube and add 2 drops of a freshly prepared solution of ferrous sulphate. Wave the tube gently back and forth in the flame until it comes to a boil, or until the brown films of ferric hydroxide appear on the side of the tube.

Cool by holding the tube under the tap water; then add dilute sulphuric acid, drop by drop, until the ferric hydroxide dissolves. If the acid solution is distinctly blue, nitrogen is present.

Write answers to the following questions in your notebook:

a. What is accomplished by heating the nitrogenous compound with sodium?

b. Why is sodium hydroxide solution added to the solution of the melt?

c. What is accomplished by the digestion with ferrous sulphate?

d. Why is oxidation of the iron salt permitted?

e. Write all equations involved.

3. Tests for Halogens. *First method. Beilstein's test.* Loop a piece of copper wire and heat the looped end in a Bunsen flame until the flame is no longer colored green.

A little of the substance to be tested, e.g., chloroform, is placed on it and is heated again. Explain the phenomena observed.

If this test yields a positive or doubtful result, try the following method:

Second method. Decompose some of the substance to be tested for halogens by means of sodium in the same manner as described in the test for nitrogen. In this case make the solution just acid with nitric acid, bring to a boil and allow to cool. Divide the solution into three parts. To one add a few drops of silver nitrate solution. To the second add ½ c.c. of carbon bisulphide and shake; then add 2 drops of chlorine water and shake again. To the third portion add a little carbon bisulphide and potassium nitrite solution and shake.

Write answers to the following questions:

a. What products are formed by the action of sodium upon the substance?

b. Why is the solution acidified with nitric acid and boiled?

c. How much (and how little) does the formation of a precipitate with silver nitrate indicate?

d. What is the action of chlorine water upon a solution of the sodium halides. Of potassium nitrite?

e. Of what use is the carbon bisulphide?

4. Test for Sulphur. *As sodium sulphide.* Decompose a little of the substance with sodium in the same manner as in the test for nitrogen. To a little of the filtered solution add 3 drops of a 1 per cent solution of sodium nitroprusside. To another portion add 1 c.c. of a solution prepared by adding sodium hydroxide to a solution of lead acetate until the precipitate first formed just redissolves. To a third portion add ½ c.c. of silver nitrate solution. Write all reactions involved.

As sulphate. Fuse a small portion of the substance provided (dried egg albumin) in a crucible with three times its quantity of fusion mixture ($2KNO_3,Na_2CO_3$). Heat cau-

tiously at first round the edge and continue heating until all charred particles have vanished. Cool; extract with hot water, and test the filtered solution for sulphates with barium chloride in the presence of a mineral acid (HCl). Write reactions involved.

5. Test for Phosphorus. Fuse, as described for sulphur, some casein with fusion mixture. Extract the fused mass with hot water and divide the solution into two parts. To the one part add excess of nitric acid and ammonium molybdate and warm; a yellow precipitate indicates phosphoric acid; to the other part add excess of ammonia and test for phosphates with magnesia mixture. Write all equations.

CHAPTER II

PURIFICATION OF ORGANIC SUBSTANCES

6. Separation of a Compound by Precipitation and its Purification by Washing and Recrystallization. To a given solution of sodium benzoate add a slight excess of dilute hydrochloric acid. Benzoic acid is precipitated. Filter off the precipitate and wash it free from hydrochloric acid with water. If possible the filtration should be done on a Buchner funnel. Then place the substance in a small beaker and dissolve in boiling water. Use just sufficient water to dissolve it. Filter the hot solution through a folded filter paper into a clean beaker. Cover the beaker with a watch-glass and allow to cool. Pure benzoic acid will crystallize out. Filter off the crystals, wash them with a little cold water, and then dry the crystals thoroughly between sheets of filter paper. Test the purity of your product by estimating its melting-point. (See Exp. 7.)

7. Determination of the Melting-point of Solids. Pulverize your own product of benzoic acid and introduce a small quantity, sufficient to occupy a length of 1 cm., into a melting-point tube. This consists of a thin-walled glass tube about 1 mm. in diameter and 5–6 cm. long and closed at one end. It is made by heating a dry piece of glass tubing and when soft drawing it out; the long capillary tube thus made is cut into the required lengths and one end is sealed by holding it in the flame. Attach the melting-point tube, when filled, by a narrow rubber band to a thermometer. Suspend the thermometer by means of a clamp in a beaker of concentrated sulphuric acid or cottonseed oil which is placed upon a wire gauze or sand-bath on a tripod. Gradually heat the beaker and at the same time stir the liquid with a glass stirrer. Note the temperature at which the substance melts.

NOTE.—A pure solid organic compound melts sharply at a definite temperature. An impure organic compound does not melt sharply and it melts at a lower temperature than a pure compound. The melting-point of a compound helps in its identification.

8. Separation of Two or More Solids by Means of Selective Non-miscible Liquids. If two solids, for example, are mixed together, and we desire to separate them, the task is easy, provided one is soluble in a liquid which is non-miscible with another liquid in which the other solid is soluble.

To separate succinic acid and urea: Place an aqueous solution of succinic acid and urea in a separatory funnel and add about one-quarter its volume of ether. Shake up the two solvents. (An increase in pressure usually occurs when ether and water and air are first shaken together in a separatory funnel. While shaking, therefore, the top of the funnel must be occasionally opened after allowing the liquids to settle at the other end.) After thoroughly shaking, allow the liquids to separate from one another, remove the stopper from the funnel and run the heavier liquid (water) into a clean beaker. Then pour the ether layer into a clean basin and allow it to evaporate.

Extract the aqueous layer two or three times with fresh quantities of ether. Combine the ethereal extracts and allow them to evaporate. Succinic acid will be obtained. Recrystallize it from hot water and determine its melting-point.

Evaporate the aqueous solution on the water-bath; urea remains. Recrystallize it from hot water and determine its melting-point.

9. Purification of Liquids by Distillation. A pure liquid has a constant boiling-point; hence, by distilling a mixture and carefully watching the boiling-point, we can separate one liquid from a mixture of two or more liquids, and by redistilling the distillate that comes over at approximately the right boiling-point we can obtain a comparatively pure product.

Make a mixture of 200 c.c. each of common commercial alcohol and water. Determine the specific gravity of the mixture, and by means of hydrometers or alcoholometers determine the percentage content of alcohol. Transfer the liquid to a distilling flask (a round-bottom flask with a side-tube in its neck), filling it half or at most two-thirds. Close the neck with a cork which carries a thermometer and adjust it so that the bulb of the thermometer is just below the opening of the side tube and not touching the walls. Connect the side tube by means of a cork to a clean, dry condenser, and place a receiver (flask) at the other end of the condenser. Place the distilling flask on a water-bath, gently heat the liquid and observe the thermometer. The mercury rises rapidly at first and then becomes stationary at a definite temperature. This is the boiling-point of the liquid. Pure alcohol boils at 78.5° C. In a mixture of alcohol and water the first fixed boiling-point will be from about 78 to 80° C. Collect the distillate as long as the thermometer remains between these two points. As soon as the thermometer rises above 80° C., remove the receiving flask; we shall call this distillate *A*. If it is desired to get all the alcohol, adjust another receiving flask and continue the distilling of the mother liquor. To obtain pure alcohol from distillate *A*, add several lumps of fused calcium chloride and allow it to stand for 12 to 24 hours. The calcium chloride has a great affinity for water and will remove it from the alcohol. Filter this liquid into a small dry, clean distilling flask and distil again. This time most of the liquid should distil over at 78.5° C. To ascertain whether any water still remains in the alcohol, transfer a small amount to a test-tube and add about $\frac{1}{2}$ gram of fused copper sulphate. Fused copper sulphate is a dirty white in color, but if it comes in contact with water it will become blue. Prove this latter by mixing $\frac{1}{2}$ c.c. of water with 4 c.c. of alcohol and then adding $\frac{1}{2}$ gram of fused copper sulphate.

CHAPTER III

THE ALIPHATIC HYDROCARBONS

A. The Paraffins

10. Preparation and Properties of Methane. Mix together 2 grams of fused sodium acetate and 6 grams of soda lime. Place the mixture in a test-tube and heat. Marsh gas is evolved and may be ignited at the mouth of the test-tube (dry distillation).

11. Fractional Distillation of Kerosene. Place about 50 c.c. of refined petroleum in a small, dry, distilling flask; connect it with a condenser and distil. Notice that the temperature indicated by the thermometer never remains constant for any length of time. This shows that the liquid is a complex mixture. Collect several fractions boiling within 10° ranges of temperature in separate receivers. By redistilling these fractions in the same way, pure products may eventually be obtained.

12. Inflammability of Kerosene. To about 3 c.c. of refined petroleum in a porcelain dish place a lighted match. What happens? Now warm the liquid on a water-bath to 40° C. and again apply a lighted match. Now what happens?

13. Inertness of the Saturated Hydrocarbons. Shake up some petroleum ether with:

A. Some concentrated sulphuric acid.

B. Some concentrated nitric acid.

C. Some sodium hydroxide solution.

Repeat the above experiment using paraffin wax instead of petroleum ether.

What happens in each case?

What do you conclude in regard to the paraffins from these experiments?

B. The Unsaturated Hydrocarbons

14. Preparation of Ethylene. C_2H_4. Set up an apparatus consisting of a 1-liter flask, a condenser, two gas wash bottles, and a pneumatic trough, making the connections as follows: Select a snugly fitting cork for the flask, and bore two holes in it. Into one hole insert a thermometer so that it will come within an inch of the bottom of the flask. Into the other hole insert a bent glass tube. Connect this tube with the condenser, which in turn is attached to a tube which passes just through the stopper of the first gas wash bottle. Connect the latter with the second wash bottle by means of a bent glass tube which reaches nearly to the bottom of each.

Pass another tube from just below the stopper of the second wash bottle to the pneumatic trough. The first wash bottle is to be left empty, and the second one is to be half filled with a concentrated solution of sodium hydroxide. This arrangement is necessary to prevent back-suction of the alkali into the hot acid solution.

When all the apparatus has been carefully adjusted, pour 30 c.c. of absolute alcohol into the flask, add a spoonful of sand, and then add, in small quantities at a time and with constant shaking, 100 c.c. of concentrated sulphuric acid. If the mixture should become very warm during this process, cool the flask under running water before adding more acid. Finally, connect the flask with the condenser and heat cautiously over a wire gauze until the temperature reaches 180°. Adjust the flame so that this temperature may be kept fairly constant. When a sample of gas collected in a test-tube over water burns readily, fill several 250 c.c. wide-mouthed glass-stoppered bottles with the gas.

Of what use was the sodium hydroxide solution in the wash bottle?

15. Properties of Unsaturated Hydrocarbons. *a. Inflammability of ethylene.* Turn one of the bottles mouth upwards, and stopper it with a cork containing two holes.

In one hole insert a short glass tube, nearly closed at the outward end. In the other hole insert a tube long enough to extend to the bottom of the bottle. Connect this tube, by means of a piece of rubber tubing, to a water bottle, and allow a stream of water to flow in slowly. At the same time ignite the gas which escapes from the other tube. Note the luminosity of the flame.

b. Action of bromine on ethylene. Pour 1 c.c. of bromine water into one of the bottles. Close the bottle with a glass stopper, and agitate the contents. Note the disappearance of the red color, and the appearance of oily drops on the sides of the bottle. On opening the bottle, notice the characteristic odor of the new compound. What is this substance? Write the equation of the reaction.

c. Von Baeyer's test for the double bond. Into another of the bottles of ethylene pour 2 c.c. of very dilute potassium permanganate solution and 1 c.c. of a 5 per cent sodium carbonate solution. Stopper the bottle with a glass stopper and shake it. Note the changes in color. What new compound is produced from the ethylene? Write the equations of the reaction.

d. Unsaturated compounds in illuminating gas. By means of a rubber tube attached to a gas jet, fill two bottles with gas by displacement of water. Repeat experiments *b* and *c*. Are unsaturated substances present?

e. Action of bromine on amylene. Dissolve 1 c.c. of amylene in 5 c.c. of carbon tetrachloride in a test-tube, and add slowly and with constant shaking, a 1 per cent solution of bromine in carbon tetrachloride. Does the red color disappear?

f. Action of oxidizing agents on amylene. Mix 10 drops of amylene with 5 c.c. of a 5 per cent solution of sodium carbonate. Then add, drop by drop, with constant shaking, a 1 per cent solution of potassium permanganate. What changes occur?

16. Preparation and Properties of Acetylene. C_2H_2. Support a dry 300-c.c. distilling flask by means of a tripod

and a clamp. Connect the side-arm of the flask with a bent glass tube leading just through the stopper of an empty bottle. Insert another bent glass tube through the stopper, to the bottom of this bottle, and connect it with a bent delivery tube which dips under water. Adjust a dropping funnel in the neck of the flask by means of a hole in the stopper. Place about 10 grams of calcium carbide in the flask, and allow water to enter from the dropping funnel, *drop by drop.* Collect four or five 250-c.c., glass-stoppered, wide-mouthed bottles of gas. Discard the first one, as it contains impurities.

a. With the acetylene gas collected in the other bottles, perform the tests described in experiment 15, *a*, *b*, and *c*.

Record your results. Write all equations.

b. Test for the triple bond. Prepare an ammoniacal solution of cuprous chloride as follows: Dissolve 3 grams of copper oxide in 10 c.c. of concentrated hydrochloric acid and 10 c.c. of water. As soon as the oxide has dissolved add 3 grams of copper filings and boil the mixture until the solution becomes colorless.

Cool, and then decant off about one-fourth of the liquid and render it alkaline with ammonium hydroxide. Pour 5 c.c. of this liquid into one of the bottles containing acetylene gas, and shake. A red precipitate of copper acetylene, C_2Cu_2, is formed. To a small portion of this precipitate add a drop or two of HCl and notice the odor of acetylene.

CHAPTER IV

HALOGEN DERIVATIVES OF THE HYDROCARBONS

16a. Preparation of Ethyl Bromide. Place a distilling flask of about 500 c.c. capacity on a sand-bath, which in turn is placed on a tripod. Close the flask with a well-fitting cork, and attach the side arm to a condenser. At the receiving end of the condenser attach an adapter (a bent tube wide at one end and narrow at the other). Immerse the long, narrow end of the adapter in water contained in an Erlenmeyer flask of about 250 c.c. capacity, which is cooled by standing in cracked ice. Mix 27 c.c. (50 grams) of concentrated sulphuric acid with 38 c.c. (30 grams) of absolute alcohol in the distilling flask and cool to room temperature, and then add 50 grams of potassium bromide. Set the flask on the sand-bath, connect with the condenser and heat. On boiling, the contents froth up, and heavy oily drops of ethyl bromide collect under the water in the receiver. If the frothing becomes excessive, remove the flask from the sand-bath for about a minute. Continue the heating as long as oily drops distil over. Place the contents of the receiver in a separatory funnel and collect the lower layer. Purify the product by returning it to the separatory funnel and shaking it with a dilute solution sodium carbonate. Withdraw the ethyl bromide, as before, and again shake up with water. Collect the ethyl bromide in a dry flask and add a few lumps of anhydrous calcium chloride to remove the traces of water which remain. Finally place the product in a small dry distilling flask, which should be fitted with a thermometer and placed on a sand-bath. Attach the flask to a dry condenser and distil. B.p. of ethyl bromide is 35–40°. The yield should be about 35 grams.

Write equations for all reactions.

16b. Properties of Monohalogens. (*a*) Solubility of ethyl bromide. Using 10 drops of ethyl bromide and 5 c.c. of the solvent in each test, determine its solubility in water, alcohol, ether, petroleum ether, and concentrated sulphuric acid. (*b*) Reaction of ethyl bromide with sodium. Place 5 c.c. of ethyl bromide in a dry test-tube and add a piece of metallic sodium about the size of a pea. Tightly close with a tube carrying a small drying-tube filled with fused calcium chloride. Set aside for an hour and examine. Write equation. (*c*) Reaction of ethyl bromide with silver nitrate. Mix 2 c.c. of ethyl bromide with 5 c.c. of a 5 per cent alcoholic solution of silver nitrate. Is silver bromide precipitated? (*d*) Reaction of ethyl bromide and alcoholic sodium hydroxide. Mix 2 c.c. of ethyl bromide with 10 c.c. of a freshly prepared 10 per cent alcoholic solution of sodium hydroxide. Boil for a minute or two. Write equation. Dilute with distilled water, render acid with nitric acid, and add silver nitrate solution. Is a precipitate produced? Compare with the previous experiment.

17. Preparation of Chloroform. Mix 50 grams of bleaching powder and 300 c.c. of water in a liter flask. Connect the flask, by means of a bent tube, to a condenser and receiver. Also suspend a dropping funnel through the cork. Into the funnel pour 10 c.c. of acetone and 40 c.c. of water, run this into the flask *drop* by *drop*, shaking frequently. As soon as you start running in the acetone, heat the mixture carefully (if the frothing becomes too violent, remove the flame for a few seconds), and continue to heat as long as droplets of chloroform appear with the water in the receiving flask. Transfer the distillate to a separatory funnel, and shake with several portions of water. Carefully run the chloroform into a dry flask, add fused calcium chloride, cork, and let it stand until the next period. As the yield is small, it need not be redistilled.

Write all reactions involved.

18. Properties of Chloroform. *a. Solubility.* Determine the solubility of chloroform in water, alcohol, ether, ben-

zene, and petroleum ether. Use about $\frac{1}{2}$ c.c. of chloroform and 5 c.c. of the solvent in each test.

b. Solvent Powers of Chloroform. Into each of 5 test-tubes place 5 c.c. of chloroform and shake up with about $\frac{1}{2}$ gram of each of the following: 1. Butter. 2. Paraffin. 3. Cottonseed oil. 4. Hard-boiled egg-yolk. 5. Iodine. Record your results.

c. Inflammability of Chloroform. Place a few c.c. of chloroform in an evaporating dish and apply a match to it. Does it burn? Heat the chloroform to the boiling-point and try to ignite the fumes.

Record your results.

d. Action of Sodium Hydroxide on Chloroform. Heat about 10 drops of chloroform with 5 c.c. of a 1 per cent solution of sodium hydroxide (free from chlorides) until the double layer disappears. Test 1 c.c. of the solution for chlorides. Nearly neutralize the rest of the solution with hydrochloric acid. Add a little mercuric chloride solution, and warm the mixture. Reduction to mercurous chloride and then to metallic mercury, indicates the presence of dissolved formate.

Write all equations.

e. Action of Silver Nitrate on Chloroform. Agitate 1 c.c. of chloroform with 10 c.c. of distilled water. Decant off the water and repeat the washing three or four times. Traces of impurities, if present, are thus eliminated. A small quantity of water and a few drops of silver nitrate solution are then added. Do you get a precipitate?

f. Tests for Impurities in Chloroform. 1. Free Chlorides. Agitate a small quantity of the specimen with about 5 c.c. of distilled water and a few drops of silver nitrate solution. Pure chloroform gives no reaction.

2. Aldehydes or Formic Acid. Heat the chloroform-silver nitrate mixture. Blackening of the precipitate, which may form, indicates the presence of aldehyde or formic acid.

3. Alcohol. The presence of ethyl alcohol may be

detected as follows: Agitate 2 c.c. of the specimen with 10 c.c. of distilled water. Filter through a wetted filter paper. Test the filtrate for alcohol by the iodoform reaction, i.e., add 2 c.c. of a 3 per cent solution of iodine in potassium iodide and 10 drops of N/2 sodium hydroxide solution. Shake this mixture and then warm the tube by placing it in a water-bath, which is kept at 70° C. (See Exp. 22, c.)

Write the changes that take place when moist chloroform is exposed to air and light.

19. Preparation of Iodoform. Dissolve 10 grams of potassium carbonate in 75 c.c. of water, add 15 c.c. of alcohol. Warm on a water-bath to about 70° C. Add, in-small quantities at a time, 5 grams of iodine. Stir the mixture after each addition. Finally add more potassium carbonate, if any color of iodine remains. After all the iodine has dissolved, cool, filter, and wash with water. Dissolve the iodoform in a few c.c. of warm alcohol, and purify by recrystallization. Examine the crystals under the microscope, filter and dry between filter papers. Make a sketch of the crystals in your note-book. Test the solubility of your iodoform crystals in ether, alcohol and water.

What is produced on boiling iodoform with an aqueous solution of sodium hydroxide?

CHAPTER V

ALCOHOLS

20. Preparation of Ethyl Alcohol by Fermentation. Dissolve 20 grams of commercial glucose in 100 c.c. of water and add 10 c.c. of a nutrient solution. (The yeast requires the presence of certain inorganic salts for its nutriment. A suitable solution may be prepared by dissolving 10 grams each of potassium nitrate, magnesium chloride, calcium nitrate, and potassium phosphate in water and diluting to 1 liter.) Place the solution in a flask which it will fill almost to the top, and which is provided with a Bunsen valve. (A Bunsen valve consists of a short piece of glass tubing passing through the cork and connected at its outer end with a piece of small rubber tubing about 2 inches long, whose other end is closed by means of a piece of glass rod. In the rubber tubing is a longitudinal slip about half an inch long which permits the egress but not the entrance of gases.) Finally macerate half a yeast cake with water, and add the resulting paste to the solution. Stopper the flask tightly and allow the mixture to stand until the next exercise. Finally, distil off 5 c.c. of the fermented sugar solution, and make the iodoform test for alcohol in the distillate.

21. Preparation of Absolute Alcohol. Put 100 c.c. of 95 per cent alcohol in a perfectly dry flask. Add 40 grams of fused calcium oxide in small lumps and tightly stopper the flask. Allow the mixture to stand until the next exercise. Decant off and distil the alcohol which has been dried over calcium oxide, using a perfectly dry condenser and receiver. Test the distillate for water with anhydrous copper sulphate, noting the time necessary for the appearance of a blue coloration. Prepare the anhydrous copper sulphate by heating two or three grams of pulverized crys-

talline copper sulphate carefully in an evaporating dish until it turns white. Avoid overheating, since this causes the formation of black copper oxide.

22. Properties of Ethyl Alcohol. *a. Action of metallic sodium.* To 10 c.c. of absolute alcohol in a small flask add about 0.5 gram of metallic sodium. Observe that effervescence occurs, just as it does when metallic sodium is added to water but that the reaction is not nearly so violent. Collect the gas evolved in an inverted tube and show that it is hydrogen by burning it. When the sodium has dissolved, evaporate the solution to dryness on a water bath. A white solid—sodium ethylate or sodium alcoholate—remains, which is very hydroscopic, and is decomposed by water, yielding alcohol and sodium hydroxide. Dissolve a little of your sodium alcoholate in a few c.c. of water. Neutralize carefully with hydrochloric acid; then evaporate to dryness. Ignite, cool and test the residue.

What is this residue?

Write all equations involved.

b. Oxidation. To a little dilute alcohol in a test-tube add a few drops of potassium bichromate solution and a little dilute sulphuric acid; warm. Note the characteristic odor of aldehyde.

Observe that the solution turns green. Why?

Write reactions involved in the above oxidation.

c. Iodoform test for ethyl alcohol. To 1 c.c. of alcohol add 5 c.c. of water, then add 2 c.c. of a 3 per cent solution of iodine in potassium iodide and 10 drops of a 5 per cent sodium hydroxide solution. After shaking, warm the mixture for a few seconds in a beaker of water heated to about 70° C.

Why is it not advisable to heat the solution above 70° or to add an excess of sodium hydroxide? Compare with Exp. 19.

d. Action of phosphorus pentachloride. To a small quantity of alcohol add a few drops of phosphorus pentachloride. A vigorous action occurs and hydrochloric

fumes are evolved. This experiment must be performed under the hood.

What are the other products produced?

What *radical* is indicated by the phosphorus pentachloride reaction?

Write reactions.

23. Determination of the Quantity of Alcohol in an Aqueous Solution. A dilute alcoholic solution containing certain non-volatile impurities will be supplied for this test.

Place 200 c.c. of the alcoholic solution in a distilling flask and distil until all the alcohol has come over. This will necessitate collecting at least 100 c.c. of distillate, and the residue should be boiling steadily at about 100°. Make the distillate up to the original volume with distilled water. Determine the specific gravity with a hydrometer, and from these data calculate the percentage by volume of alcohol in the original mixture.

Define percentage by volume and percentage by weight.

23a. Tests for Methyl Alcohol. 1. Heat a small quantity of the solution to be tested with a little potassium bichromate and strong sulphuric acid. The development of the pungent odor of formaldehyde indicates that methyl alcohol was present. Are the fumes acid, and if so, why?

2. Take a piece of copper wire about a foot long and twist one end of it into a spiral by winding it about a glass stirring rod. Heat the spiral to redness for a couple of minutes. Cool. Note that it is nearly black due to the oxidizing of the copper. Again heat the spiral to redness and immediately plunge it into a test-tube containing about 5 c.c. of the liquid supposed to contain methyl alcohol. The copper oxide is reduced to metallic copper (note change of color). Can you detect the odor of formaldehyde? In case the odor of formaldehyde is not clearly distinguishable? apply the resorcinol test for formaldehyde to the solution in the test-tube. (See Exp. 32.)

3. United States Pharmacopœia Test for Methyl Alcohol in the Presence of Ethyl Alcohol. This method is reliable

for the detection of 2 per cent or more of methyl alcohol in ethyl alcohol. Dilute the liquid so that it will contain about 10 per cent of total alcohols. Introduce some of this diluted alcohol mixture into a test-tube and place it in a beaker of cold water. Oxidize it by four or five applications of the hot copper spiral (see the above experiment). Filter and boil until it is free from any odor of acetaldehyde. Cool. Add 1 drop of a 0.5 per cent resorcinol solution and pour the resulting mixture slowly, so that the two liquids do not mix, down the sides of an inclined test-tube containing about 5 c.c. of concentrated sulphuric acid. Allow the tube to stand for three minutes, after the end of which time, gently rotate it. If a rose-red ring appears at the junction of the two liquids, 2 per cent or more methyl alcohol is present. Acetaldehyde gives with the resorcinol test a yellowish-brown ring and a flocculent precipitate, hence the advantage of expelling it from the solution.

CHAPTER VI

THE ETHERS

24. Preparation of Ethyl Ether by the Continuous Process. A distilling flask of about 300 c.c. capacity is fitted with a cork carrying a tap funnel and a thermometer, both arranged so as nearly to touch the bottom. After 50 c.c. of 95 per cent ethyl alcohol and 50 c.c. of concentrated sulphuric acid have been cautiously mixed in the flask, 5 grams of anhydrous aluminium sulphate are added, and the flask is connected to a long condenser. An adapter is fitted to the lower end of the condenser and passes through the neck of a suction flask, which is surrounded by ice and has a piece of rubber tubing attached to its side arm. The rubber tubing is arranged so as to carry any volatilized ether into the waste pipe of the sink. Heat is then applied until a temperature of 135 to 140° is attained. As soon as the distillation of ether begins, alcohol is introduced from the tap funnel at the same speed as the liquid distils (about 3 drops a second), and the distillation continued with a small flame so as to maintain a constant temperature. After about 100 c.c. of distillate has been obtained it is agitated in a separatory funnel with an equal volume of water, which dissolves most of the admixed alcohol and sulphurous acid, leaving a layer of ether floating on the surface of the solution. After the ether is separated from the aqueous liquid, it is dried by agitation for about ten minutes with fused, powdered calcium chloride.

How would you test for the presence of water in a given sample of ether?

Write reactions involved in the preparation of ether by this process.

How else could ether be made?

How would you make a mixed ether?

25. (**Demonstration.**) **Preparation of Anhydrous Ether.** Ether obtained by a method similar to that indicated in Exp. 24 contains traces of alcohol and water. These traces can be removed only by treatment with metallic sodium.

The ether is placed in a flask provided with a calcium chloride tube to prevent the access of moisture, and to allow the escape of hydrogen; several pieces of freshly cut metallic sodium are added and the mixture is repeatedly agitated until no further effervescence is observed. The ether is decanted into a perfectly dry distilling flask and distilled from a water-bath. Pure ether of constant boiling-point (35°) is collected.

Properties of Ether

26. Miscibility of Ether with Other Liquids. Into each of six perfectly clean, dry test-tubes place 5 c.c. of ethyl ether, then mix with an equal volume of the following: Alcohol, glycerol, benzene, chloroform, petroleum ether, and water.

Record your results.

27. Solvent Power of Ether. Add about 5 c.c. of ethyl ether to each of five test-tubes containing a lump about the size of a pea of (1) lard, (2) paraffin, (3) hard-boiled egg-white, (4) hard-boiled egg-yolk, (5) bread crumbs. Warm each test-tube by holding it in your hand for a couple of minutes; then if you have any doubt in regard to the solubility of the substance, filter the supernatant ether through a dry filter paper onto a clean dry watch-glass and ascertain if any residue remains after the ether has evaporated.

Record your results.

28. Absorption of Heat Caused by Evaporation of Ether. Demonstrate that the evaporation of ether causes absorption of heat by placing a small quantity in the palm of the hand, and blowing on it. A sensation of cold is felt immediately.

29. Inertness of Ether Towards Chemical Reagents. Into each of 5 test-tubes place 5 c.c. of *anhydrous* ether and shake up with the following:

a. 2 c.c. of phosphorus pentachloride.
b. 2 c.c. of 10 per cent sodium hydroxide solution.
c. 2 c.c. of 10 per cent alcoholic soda.
d. 2 c.c. of concentrated sulphuric acid.
e. A lump the size of a pea of metallic sodium.

What happens in each case?

What do you conclude in regard to the chemical activity of ethers from these experiments?

CHAPTER VII

THE ALDEHYDES AND KETONES

30. Preparation of Formaldehyde. *a.* Heat a few drops of methyl alcohol with potassium bichromate and sulphuric acid. Note the pungent, suffocating odor of formaldehyde. Hold a piece of blue litmus paper in the fumes.

What do you observe? Why?

b. Take a piece of fine copper wire about a foot long and twist one end of it into a spiral by winding about a glass stirring rod. Heat the spiral to redness for a couple of minutes. Cool. Note that it is nearly black due to the oxidizing of the copper. Heat the spiral again to redness and at once plunge it into a test-tube containing about 2 c.c. of methyl alcohol. The copper oxide is reduced to metallic copper (note change of color). The vapors of formaldehyde are especially noticeable.

Ascertain if the fumes are acid.

Write the equations for the reactions.

31. Detection of Formaldehyde in Milk. (Leach's method.) Put 10 c.c. of the milk to be tested into a casserole or small porcelain dish and add 10 c.c. of concentrated hydrochloric acid which contains one drop of a 5 per cent solution of ferric chloride. Hold the casserole just above a small Bunsen flame, and keep it moving in a rotary fashion until the liquid just reaches the boiling-point. Should formaldehyde be present, a violet color, more or less pronounced, will appear when the temperature reaches 80° or 90°. In the absence of formaldehyde, the liquid will assume a turbid yellowish-brown hue.

32. The Resorcinol Test for Formaldehyde. To about 2 c.c. of *very dilute* formaldehyde solution (or solution suspected of containing a small amount of formaldehyde), add

one drop of a 1 per cent aqueous solution of resorcinol and shake thoroughly. Pour this liquid cautiously into a second test-tube containing about 3 c.c. of concentrated sulphuric acid. If the second test-tube is properly inclined the mixture will form a distinct layer upon the surface of the acid.

A red zone slightly violet in color will appear, and above the zone there will be a light flocculent precipitate. This reaction is characteristic of formaldehyde; other aldehydes do not show this behavior.

33. (Demonstration.) Action of Formalin on Proteins. The yolk of an egg is carefully placed in formalin (a 40 per cent solution of formaldehyde in water). On allowing to stand for some time, it will be found to have acquired the consistency of a rubber ball.

34. Preparation of Acetaldehyde. In a 500-c.c. distilling flask place 20 c.c. of 95 per cent ethyl alcohol, 100 c.c. of dilute sulphuric acid (1 : 10), and 40 c.c. of a 10 per cent solution of potassium bichromate, attach to a condenser so that the receiving end dips just under the surface of about 10 c.c. of cold water. Distil until 15 or 20 c.c. have been collected. This distillate may be used to perform the aldehyde reactions described in Exps. 35, 36, 37, 39, 40, 41 and 42.

Properties of Aldehydes

35. Silver Mirror Test. To a little silver nitrate solution add dilute ammonium hydroxide solution until the precipitate which first formed just redissolves. Then add a few drops of dilute aldehyde solution (if the distillate obtained in Exp. 34 is used, first dilute a small portion of it with water), place in a water-bath containing cold water and heat to boiling. A mirror of metallic silver forms on the glass.

Note.—To obtain good results the test-tube used in this experiment must be *perfectly* clean. The best way to clean the test-tube is to boil a little sodium hydroxide in it, and then to wash several times with water.

36. Reduction of Fehling's Solution. To a little mixed Fehling's solution add a few drops of dilute aldehyde solution and warm. A red or yellow precipitate of cuprous oxide is formed. Explain these changes.

NOTE.—Fehling's solution consists of copper sulphate, caustic soda, and Rochelle salt (sodium potassium tartrate). When caustic soda is added to copper sulphate a blue precipitate of cupric hydrate, $Cu(OH)_2$, is formed, which turns black on boiling. The presence of the Rochelle salt keeps the $Cu(OH)_2$ in solution, and forms a deep blue solution. This solution does not keep, so that it must be freshly made for each experiment. For this purpose the two solutions must be kept separate. One contains the copper sulphate, the other the Rochelle salt and caustic soda. When required for use, equal parts of each are mixed together.

37. Formation of Aldehyde Resin. To a little acetaldehyde solution add sodium hydroxide and warm. The liquid becomes darker and a brown resinous substance, aldehyde resin, separates out, with the evolution of a peculiar smell. Aldehyde resin is insoluble in water, but soluble in alcohol and ether.

38. Repetition of Aldehyde Resin Test with Formaldehyde. Repeat Exp. 37, using formaldehyde instead of acetaldehyde. Do you get the same result?

39. Schiff's Aldehyde Reaction. To a little dilute acetaldehyde solution add a solution of magenta decolorized with sulphurous acid (Schiff's aldehyde reagent). The red color of the dye is restored.

40. Oxidation of an Aldehyde to an Acid. Mix 5 c.c. of 10 per cent potassium bichromate, 5 c.c. of dilute sulphuric acid, and a little of the aldehyde solution and warm. The solution becomes green. Why? Hold a piece of blue litmus in the steam. Does it turn red? Write reaction. If acetaldehyde is used, what acid is obtained?

41. Polymerization of Aldehydes. Place a clean test-tube, containing about 2 c.c. of acetaldehyde, in a freezing-mixture (2 parts cracked ice and 1 part sodium chloride). Then add a small drop of concentrated sulphuric acid.

This can best be done by dipping a clean stirring-rod into the acid. On stirring the thoroughly cooled aldehyde, it should finally solidify. Is the odor still like that of aldehyde? Explain this change.

42. Formation of Hydrazone. Dissolve about a gram of a mixture of phenylhydrazine hydrochloride and sodium acetate (these are mixed on a basis of 1 part of the phenylhydrazine hydrochloride to $2\frac{1}{2}$ parts of sodium acetate) in 10 c.c. of water, add 10 drops of acetaldehyde and warm in a water-bath; an oil (acetaldehyde phenylhydrazone) is formed. Write equation of the reaction.

43. Reducing Action of Chloral and Chloral Hydrate. Repeat Exps. 35 and 36, using a solution of chloral hydrate instead of the aldehyde.

What changes occur? Explain them.

Write equations showing how chloral could be prepared from acetaldehyde; from alcohol.

44. Formation of Chloroform from Chloral Hydrate. To 5 c.c. of a 50 per cent aqueous solution of chloral hydrate add about 2 grams of solid sodium hydroxide and warm gently (with hand). Identify the liquid which separates. Write equation of the reaction.

THE KETONES

45. Preparation of Acetone. Place in a round-bottom flask 50 grams of dry calcium acetate. Connect the flask with a condenser and heat on a sand-bath to a high temperature. Acetone is the principal ingredient of the distillate.

Write equation. Use this distillate in Exps. 46 to 52.

Properties of Ketones

46. Oxidation of Acetone. Mix 2 c.c. of the acetone distillate with a small quantity of very dilute sulphuric acid. Heat gently and add potassium permanganate solution, little by little, until the warm solution retains a pink

color. Filter, acidify strongly with sulphuric acid and distil. Since the quantity is small, a test-tube fitted with cork and L-shaped glass tube may be used instead of a distilling flask. Retain the distillate until after you perform the experiments on acetic acid (Exp. 56), then ascertain whether the distillate contains acetic acid.

47. Reduction of Acetone. Repeat Exps. 35, 36 and 39, using acetone, then answer the following questions: *a.* Does acetone give a positive silver mirror test? *b.* Does it reduce Fehling's solution? Does it color Schiff's reagent? What would be formed by the reduction of acetone?

48. Formation of Addition Products. Shake thoroughly a mixture consisting of 5 c.c. of a saturated solution of sodium hydrogen sulphite and 5 c.c. of the acetone distillate. Cool. Examine the crystalline deposit with a microscope.

Do all ketones and aldehydes form similar addition products with sodium hydrogen sulphite?

49. Formation of Hydrazones by Ketones. Repeat Exp. 42, using acetone instead of acetaldehyde. Does acetone form a hydrazone?

50. Sodium Nitroprusside Test. To about 5 c.c. dilute acetone solution (or solution suspected of containing acetone), add a few drops of a freshly prepared aqueous solution of sodium nitroprusside and about 2 c.c. of 8 per cent sodium hydroxide solution. A ruby red color is produced if the acetone is present. Acidify with acetic acid. Is there any change of color?

51. Salicylic Aldehyde Test. To 10 c.c. of dilute acetone solution (or solution suspected of containing acetone), add about a gram of solid sodium hydroxide, and before it dissolves introduce 10 drops of salicylic aldehyde. Warm to 70° in a water-bath. A purple-red ring appears if acetone is present.

NOTE.—If the alkali has dissolved before adding the salicylic aldehyde the liquid becomes yellow, red, and finally purplish red.

52. Iodoform Test for Acetone. To about 5 c.c. of dilute acetone solution (or solution suspected of containing acetone), add about 10 drops of sodium hydroxide and then, drop by drop, iodine solution, iodine in potassium iodide, until the liquid is faintly yellow. If acetone is present iodoform will separate at once.

Note.—Acetone yields iodoform at room temperature, whereas alcohol requires heating.

CHAPTER VIII

FATTY ACIDS

Formic Acid. Exactly neutralize about 10 c.c. of formic acid with sodium hydroxide and use the solution of sodium formate in Exp. 53 and 54.

53. Reducing Action of Formic Acid. *a.* Mix about 3 c.c. of neutral sodium formate solution with an equal volume of silver nitrate solution and warm. A black deposit of metallic silver is formed. Test the reaction of the filtrate.

Write the equations.

Explain the reducing action of formic acid.

b. To another portion of the sodium formate solution add a few drops of mercuric chloride solution, and warm gently. The white precipitate which separates is mercurous chloride ($HgCl$). Filter the solution; then moisten the precipitate on the filter paper with ammonium hydroxide. Note the change of color. Explain it.

54. Action of Concentrated Sulphuric Acid on Formic Acid. Evaporate about 5 c.c. of the sodium formate solution to dryness. Transfer the solid residue to a dry test-tube; add a little concentrated sulphuric acid and heat. What is the gas evolved? Does it burn?

Write the equation.

55. Preparation of Acetic Acid by Oxidation of Ethyl Alcohol. Ten grams of potassium dichromate are mixed with 10 c.c. of concentrated sulphuric acid in a 300-c.c. distilling flask. The flask is then connected to a condenser by its side arm and 25 c.c. of a 5 per cent aqueous solution of alcohol is added, drop by drop, from a tap-funnel. After all the alcohol has been added, the tap-funnel is closed, the flask is heated, and the distillate collected.

The presence of acetic acid in the distillate may be as-

certained by its odor, and after careful neutralization with sodium carbonate, by the ferric chloride test (Exp. 56).

56. The Basic Acetate Test for Acetic Acid. Neutralize a little dilute acetic acid with sodium hydroxide solution and add a few drops of ferric chloride. The solution turns blood-red, due to the formation of ferric acetate. Divide this solution into two parts. Boil part one, and add hydrochloric acid to part two.

Explain the changes observed.

57. Action of Sodium Acetate on Reducing Agents. Repeat Exp. 53, using sodium acetate instead of sodium formate. Does acetic acid have any reducing action?

58. Freezing-point of Glacial Acetic Acid. Immerse a small test-tube containing about 5 c.c. of glacial acetic acid in some freezing-mixture (2 parts ice, 1 part salt). Stir with a glass rod and when the mass is about half frozen insert a thermometer and note the solidifying temperature of the acid. Pure glacial acetic acid melts at 16.5°. How would the presence of water affect the freezing-point?

59. Inflammability of Acetic Acid Fumes. Apply a flame to some glacial acetic acid. Does it burn? Now heat a few drops in a test-tube and apply a flame to the vapors. Do they burn?

Chemical Properties of the Higher Fatty Acids

60. Solubility of Stearic Acid. Test the solubility of stearic acid in water, cold alcohol, warm alcohol and ether.

Record your results.

61. Formation of Soap. Suspend a small amount of stearic acid in water and add sodium hydroxide drop by drop with constant stirring; it dissolves slowly, forming a clear solution which foams considerably on agitation.

Is a sodium soap a hard or soft soap?

62. The Reaction of Soaps. Make a concentrated solution of a neutral soap. Test its reaction to phenolphthalein. Pour some of this soap into a beaker full of distilled water. Explain the changes observed.

CHAPTER IX

ACID CHLORIDES, ACID ANHYDRIDES, ESTERS AND MERCAPTANS

Properties of the Acid Chlorides (Acyl Haloids)

63. Action of Water on Acetyl Chloride. Place 5 c.c. of water in a test-tube and add about ½ c.c. of acetylchloride, drop by drop. What gas escapes? Test the reaction of the liquid that remains in the test-tube with litmus paper. Write the equation for the reaction.

64. Action of Alcohol on Acetyl Chloride. Place 2 c.c. of absolute alcohol in a small test-tube. Hold the test-tube in a beaker of cold water, then add, drop by drop, about 1 c.c. of acetyl chloride. Make the solution faintly alkaline, then pour it upon a watch-glass and notice the odor. What is formed? Write the equation for the reaction.

For the use of acyl haloids as a means of detecting the number of hydroxyl radicals in a carbohydrate see Chapter XII.

Why do the acyl haloids fume in the air?

Properties of the Acid Anhydrides

65. Action of Water on Acetic Anhydride. Mix a few drops of acetic anhydride with a little water. What is formed? How could you detect it?

66. Action of Alcohol on Acetic Anhydride. To about 2 c.c. of absolute alcohol add an equal volume of acetic anhydride, drop by drop, and warm gently in a water-bath. Add a little water to the product and make it slightly alkaline with sodium hydroxide. Pour it upon a watch-glass and notice the odor. Does it resemble the odor obtained by the action of alcohol on acetyl chloride?

Write the equation of the reaction. Do acid anhydrides fume in the air?

Esters of Inorganic Acids

67. Preparation of Ethyl Nitrite. (Sweet Spirits of Nitre.) Warm a little alcohol with copper filings and a few drops of concentrated nitric acid in a test-tube and note the odor of ethyl nitrite. Write the equation of the reaction.

68. Preparation of Potassium Ethyl Sulphate. Carefully mix 10 c.c. of concentrated sulphuric acid with 25 c.c. of absolute alcohol in an Erlenmeyer flask on a water-bath under a reflux (upright) condenser for $\frac{1}{2}$ hour. Cool and then pour into a casserole containing about 150 c.c. of water. Gradually add calcium or barium carbonate, with constant stirring, until the solution is alkaline to litmus. What salt is formed? Boil the mixture and filter. Place the clear filtrate on the water-bath, heat again, and add a strong solution of potassium carbonate until no further precipitate is formed. Allow the precipitated calcium carbonate to settle and decant the supernatant liquid onto a filter paper. Finally evaporate the filtrate to a very small volume. On cooling, crystals of ethyl potassium sulphate will settle out. Wash the crystals with dilute alcohol, dry between filter papers and keep in a small stoppered vial for future experiments.

Write all equations.

Esters of Organic Acids

69. Preparation of Ethyl Acetate. Mix 1 c.c. of absolute alcohol and 1 c.c. of glacial acetic acid in a test-tube and heat in a boiling water-bath. Note the change of odor. Write the equation of the reaction. Compare Exps. 64 and 66.

70. Hydrolysis (Saponification) of Ethyl Acetate. Place in a distilling flask 20 c.c. of ethyl acetate, 50 c.c. of a 20 per cent solution of sodium hydroxide, and a small piece of pumice stone.

Put the flask on a water bath and attach a condenser in a vertical position so that the condensation products will return to the flask ("reflux condenser"). Shake the mixture frequently to prevent bumping. After the layer of ethyl acetate has disappeared, connect the condenser in the usual manner for distillation, and collect all the liquid that distils under about 90°.

Prove the presence of ethyl alcohol in the distillate by applying the iodoform test (Exp. 22, *c*.)

The presence of acetic acid in the residue in the flask should be proven by acidifying with dilute sulphuric acid and again distilling. This distillate should be tested for acetic acid. Apply the basic acetate test. (Exp. 56.)

Write all equations.

71. Preparation of Ethyl Mercaptan. Prepare 2 to 5 c.c. of a saturated solution of ethyl potassium sulphate from the product prepared in Exp. 68. Add an equal volume of a 33 per cent solution of potassium hydrogen sulphide and warm.

Notice the garlic-like odor of the mercaptan.

What products are formed when the mercaptans are oxidized?

Write equations showing how ethyl mercaptan may be used in the preparation of the following: Sulphonal, trional, and tetronal.

CHAPTER X

AMINES, AMIDES, AND CYANOGEN COMPOUNDS

The Amines

71a. Preparation of Methyl Amine. Make a paste of bleaching powder in a 500 c.c. distilling flask by adding 20 grams of bleaching powder and 60 c.c. of water and shaking thoroughly. Render the solution strongly alkaline by the addition of freshly slacked lime. To this mixture add 15 c.c. of a 40 per cent aqueous solution of acetamide, $CH_3CO—NH_2$. Distil with steam until the distillate has only a faintly alkaline reaction. This can be ascertained by holding a piece of litmus paper from time to time in the drops that are coming over. The apparatus for steam distillation is shown in Fig. 1.

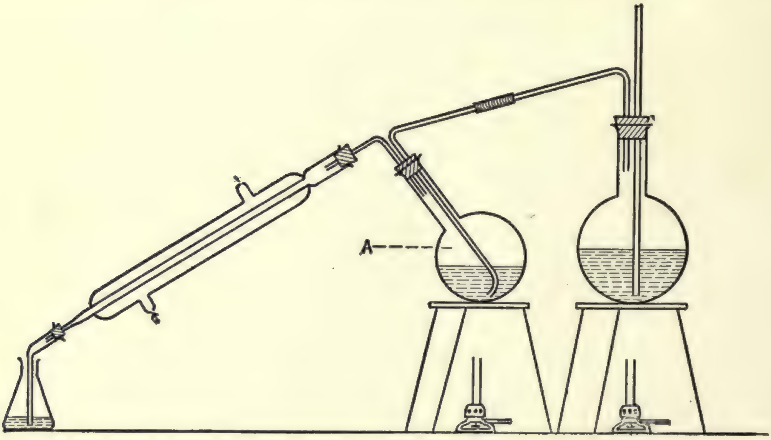


Fig. 1.—Apparatus for Steam Distillation.

A liter flask or can, such as an ether-can, may be used to generate the steam. The flask or can is fitted with a two-hole stopper. A safety-tube, i.e., a long glass tube reaching nearly to the bottom of the flask, is passed through one hole. A bent tube which terminates just below the cork is passed through the second hole, and this tube is attached by rubber tubing to the inlet tube of distilling flask A. The distilling flask must be placed in a sloping position so as to prevent splashing of its contents and the mechanical carrying over of the contents into the condenser. The inlet-tube which carries the steam is passed into the bottom of the distilling flask so that its end lies in a vertical position. To prevent condensation of steam the distilling flask is also heated. The flask is attached to a condenser by means of a bent tube. In order to prevent loss of methyl amine connect an adapter by means of a tightly fitting rubber stopper with the end of the condenser. Place 50 c.c. of distilled water in the receiver, and arrange it so that the end of the adapter will be just below the surface.

72. Properties of a Typical Primary Amine (Methylamine). *a. Reaction.* Test the reaction of some of your methylamine distillate to litmus.

b. Carbylamine Reaction. To a small portion of the methylamine distillate add a couple of drops of chloroform and 2 c.c. of alcoholic sodium hydroxide solution and warm. Note the characteristic odor of isonitriles, R–NC. Acidify the contents of the tube before pouring it into the sink.

c. Reaction with Certain Metallic Salts. To 1 c.c. of a dilute solution of copper sulphate add, drop by drop, as long as any change occurs, some of the methylamine distillate. Repeat with a dilute solution of ferric chloride. Then try the effect of ammonium hydroxide solution on solutions of these salts. Make note of all the color changes produced. Write equations for all the reactions.

d. Preparation of Methylamine Hydrochloride, $CH_3—NH_2—HCl$. Slightly acidify the remainder of the

methylamine distillate with dilute hydrochloric acid, and evaporate the mixture to dryness on the steam-bath. To dissolve and purify the salt, add to the residue in the beaker on the steam-bath about 15 c.c. of absolute alcohol, cover with a watch-glass and digest for a minute or two. Filter through a stemless funnel on a small filter paper. If all the salt does not dissolve, repeat the process as often as necessary. Cool the combined filtrates, and add an equal volume of ether. Filter on a Buchner funnel, if available. After drying, determine the melting-point of the crystals.

e. Action of Nitrous Acid on Methylamine Hydrochloride. Mix in a test-tube about ½ gram of methylamine hydrochloride, 1 gram of sodium nitrite, 1 c.c. of dilute hydrochloric acid, and 5 c.c. of water. Close the test-tube with a cork carrying a bent delivery tube suitable for collecting gas under water. What is the gas evolved? Does it extinguish a glowing splinter? Write the equation of the reaction. After the evolution of gas has ceased, or nearly ceased, arrange your apparatus for distilling liquid and collect about 2 c.c. of the distillate in a test-tube kept cool by surrounding it with cold water. Test the distillate for methyl alcohol. (See Exps. 23a, (1) and (2)).

f. Inflammability of Methylamine. Introduce about 0.5 gram of methylamine hydrochloride and 5 c.c. of sodium hydroxide solution into the test-tube. Fit the tube with a cork containing a bent tube with a small opening at the end. Heat, and ignite the gas evolved. Does it burn? Repeat this test, using ammonium chloride instead of the methylamine. Does ammonia burn?

72a. Preparation of Dimethylamine from Dimethylaniline. Dilute 10 c.c. of concentrated hydrochloric acid with 40 c.c. of water, and then dissolve 5 grams of dimethylaniline in it. Cool the mixture by surrounding it with cracked ice, and add, slowly and with constant stirring, 20 c.c. of a 15 per cent solution of sodium nitrite. Yellow nitrosodimethylaniline hydrochloride is precipitated. Allow the precipitate to settle for fifteen minutes, and then filter

by suction. Wash with dilute hydrochloric acid, and purify by crystallization from hot water. Filter. Triturate the salt with cold water and add sodium hydroxide solution. Green nitrosodimethylaniline is liberated. Transfer the mixture to a separatory funnel and shake up with 25 c.c. of ether. On evaporating the ether, the base is obtained in yellowish-green leaves. Transfer the crystals to a distillation apparatus, add a little water and some sodium hydroxide solution and distil. The *dimethylamine* will be obtained in the distillate. The residue will become dark-red owing to the production of sodium nitrosophenoxide.

73. Reactions of the Secondary Amines. Repeat the tests outlined in Exp. 72, using the dimethylamine distillate instead of the monomethylamine distillate.

Record your results.

74. Reactions of the Tertiary Amines. Repeat the tests outlined in Exp. 72, using a 5 per cent aqueous solution of trimethylamine instead of the monomethylamine distillate. Record your results.

The Amides

75. Preparation of Acetamide. Mix about 5 c.c. of ethyl acetate with an equal amount of ammonium hydroxide solution (sp. gr. 0.90), in a small flask. Place this flask in a basin of warm water (about 50° C.) and allow it to stand until the two layers form a homogeneous solution. Finally connect with a long glass tube and distil. At first the ammonia is expelled, also alcohol and water; subsequently the acetamide will distil over, and will solidify.

Write the equation of the reaction.

76. Properties of Acetamide. This experiment may be performed either with the product obtained in Exp. 75 or a purified product supplied by the instructor.

a. Action of nitrous acid. To a little acetamide solution add some sodium nitrite solution and a few drops of dilute hydrochloric acid. Note the effervescence of nitrogen gas.

Write the equation of the reaction.

Compare with Exp. 72 *b*.

b. Hydrolysis by means of an alkali. Boil a little acetamide solution in a beaker with some sodium hydroxide solution. Note the odor of the fumes. Ascertain if ammonia is given off by holding a piece of moist red litmus paper in the fumes.

c. Hydrolysis by means of heating with an acid. Boil a little acetamide solution in a beaker with a few c.c. of dilute hydrochloric acid. Neutralize with sodium hydroxide solution and test for acetic acid with ferric chloride (Exp. 56).

Write all equations of the reactions.

77. Preparation of Carbamide (Urea). Dissolve 20 grams of potassium cyanate in a small volume of water in a casserole. Add to it 20 c.c. of a saturated solution of ammonium sulphate. Evaporate to dryness on a water-bath. (The potassium cyanate first reacts with the ammonium sulphate and forms ammonium isocyanate, and then urea.) Add 25 c.c. of 95 per cent alcohol to the dry residue; stir; place on a water-bath and heat just to the boiling-point; then pour the hot solution on a small dry filter. Evaporate the filtrate to dryness on a water-bath; redissolve the residue in 15 c.c. of absolute alcohol; again filter on a small dry filter; and evaporate this filtrate *nearly* to dryness on a water-bath. On allowing the alcoholic residue to cool, crystals of urea will form.

Write all equations.

77a. Second Method for the Preparation of Urea. In case potassium cyanate is not available, the following method may be used to prepare urea.

Powder some crystallized potassium ferrocyanide finely and dry it by gently heating it on a flat iron dish (an iron sand-bath is suitable). Dry some manganese dioxide in a similar manner. Mix 25 grams of the manganese dioxide with 50 grams of the dried potassium ferrocyanide. Heat the mixture in the same iron dish, with constant stirring, until the mass glows throughout. Extract the fused mass

with a small quantity of warm water. Filter, and to the filtrate add 37 grams of powdered ammonium sulphate and shake well. On cooling, some potassium sulphate will crystallize out. To extract the urea, add 50 c.c. of methyl alcohol, heat to boiling, filter hot, and allow to cool. Recrystallize by repeating this process once with methyl alcohol, and finally with absolute ethyl alcohol. The product should now be pure. Yield 8–10 grams.

78. Reactions of Urea. *a. Solubility.* Test the solubility in water, cold alcohol, and ether. Use small quantities of solute and solvent.

b. Formation of urea nitrate. To a drop of a concentrated aqueous solution of urea add a drop of strong nitric acid. Crystals of urea nitrate are formed. Examine them under a microscope. What shape are they?

c. Formation of urea oxalate. To a drop of a concentrated aqueous solution of urea add a drop of a saturated solution of oxalic acid. Crystals of urea oxalate are formed. Examine these under the microscope. What shape are they?

d. Formation of biuret. Heat some dry crystals of urea to a high temperature in a test-tube. Note carefully what takes place as the heating is continued. Determine the reaction of the vapor produced.

Let the test-tube cool. Examine the residue. Does it resemble urea in appearance?

The white residue consists principally of biuret, but contains also cyanuric acid. Dissolve some of the solid mass in a few c.c. of water, add an equal volume of sodium hydroxide solution, and one drop of a 1 per cent copper sulphate solution. Note the pinkish color that forms (biuret reaction).

e. Repeat the biuret reaction with an aqueous solution of unheated urea. Does it act the same?

f. Action of nitrous acid on urea. To a few c.c. of an aqueous solution of urea in a test-tube add a little dilute hydrochloric acid and one or two drops of sodium nitrite solution. An effervescence of nitrogen and carbon dioxide takes place.

Write the equation of the reaction.

g. Action of an alkali on urea. Add a little sodium hydroxide solution to some urea in a test-tube and heat to boiling. What is the reaction of the vapors?

Write the equation of the reaction.

The Cyanogen Compounds

79. Preparation of Cyanogen. Heat a small quantity of mercuric cyanide in a dry test-tube containing a cork through which projects a small, bent, glass tube. Note that white fumes are given off which condense on the cooler parts of the test-tube and tube. On igniting the gas which is given off, it will burn with a characteristic purple flame.

80. Preparation of Hydrocyanic Acid (Prussic Acid). (Demonstration). 10 c.c. of a cold saturated solution of potassium ferrocyanide and 20 c.c. of a 20 per cent solution of sulphuric acid are placed in a small distilling flask, which is immediately connected with a condenser. The receiving end of the condenser is dipped into a test-tube containing a 1/10 normal solution of sodium hydroxide. On heating hydrocyanic acid distils, which is converted into sodium cyanide. Hydrocyanic acid is so excessively poisonous that it is advisable to take these precautions. The reactions of the salts are analogous; hence, they will be used in studying the properties of this compound.

81. Reactions of Hydrocyanic Acid. *a. Prussian-blue test.* To a little of the solution obtained in Exp. 80, or to a 1 per cent solution of potassium cyanide, plus an equal volume of a 1 per cent solution of sodium hydroxide, add a little ferrous sulphate and boil for a few seconds. On acidifying this solution and adding a drop or two of ferric chloride, a precipitate of Prussian-blue is formed.

Write the equation of the reaction.

b. Hydrolysis induced by boiling. Boil about 20 c.c. of a 1 per cent solution of potassium cyanide. It is hydrolyzed into potassium formate and ammonia. Test the vapors with red litmus.

CHAPTER XI

LIPINS

When animal or vegetable tissues are extracted with ether, hot alcohol or certain other organic solvents, the soluble material consists of common fat together with a variable amount of other substances, which have more or less properties in common with the fats. Some of these substances, like fats, yield fatty acids on hydrolysis, but differ from neutral fat in that they contain nitrogen or nitrogen and phosphorus in the molecule. Others have no chemical resemblance to fat, but are soluble in the fat solvents and have a greasy feel. These and other reasons justify our classifying them together. The following partial classification of the lipins, based on that proposed by Gies and Rosenbloom will give a general idea of these bodies:

Lipins. Alcohol-ether soluble constituents of tissues, which are insoluble in water and have a greasy feel.

- *A. Fats.* Neutral esters of fatty acids and glycerol which are solid at 20° C.
- *B. Fatty oils.* Neutral esters of fatty acids and glycerol which are liquid at 20° C.
- *C. Fatty acids.*
- *D. Sterols.* Alcohols, generally of the terpene group, solid at ordinary temperatures; e.g., cholesterol and phytosterol.
- *E. Waxes.* Esters of sterols and fatty acids; e.g., beeswax.
- *F. Phospholipins.* Phosphatides. Substances containing fatty acids, nitrogen and phosphorus; e.g., lecithin and cephalin.
- *G. Glycolipins.* Substances containing fatty acids, nitrogen and a carbohydrate group, but no phosphorus. The cerebrosides. (Thudicum.) Phrenosin, kerasin and cerebron.

The Fats. Neutral esters of glycerol and fatty acids.

82. Solubility. Test separately the solubility of lard and cottonseed oil in each of the following solvents: Water, cold alcohol, hot alcohol, ether, chloroform, and petroleum-ether.

Record your results.

83. Formation of Crystals. Dissolve about a gram of lard in a little ether in a test-tube and add an equal volume of alcohol. Pour the solution into a clean dry watch-glass; set in a quiet place; and allow the solvents to evaporate spontaneously. Examine the residue under a microscope.

Does it show crystalline formation?

84. Reaction. Dissolve a few c.c. of *fresh* cottonseed oil in a little alcohol, and then test its reaction to litmus, to Congo red, and to phenolphthalein.

Repeat the test, using *rancid* cottonseed oil.

Record your results and explain them.

85. Emulsification. Prepare 6 test-tubes as follows:

1. Ten c.c. of water and 2 c.c. of neutral cottonseed oil.
2. Ten c.c. of water, 2 drops of 5 per cent NaOH, and 2 c.c. of neutral cottonseed oil.
3. Ten c.c. of water, 2 drops of oleic acid, and 2 c.c. of neutral cottonseed oil.
4. Ten c.c. of water, 2 drops of 5 per cent NaOH, 2 drops of oleic acid, and 2 c.c. of neutral cottonseed oil.
5. Ten c.c. of water, 2 drops of 5 per cent NaOH, and 2 c.c. of rancid cottonseed oil.
6. Ten c.c. of water, 2 drops of 5 per cent NaOH, and 2 c.c. of commercial olive oil.

Shake each thoroughly for one minute; set aside; examine at the end of 5, 15, 30 and 60 minutes; record your observation; then answer the following questions:

What sort of an emulsion do you get in each case, and why?

What is an emulsion? What is a temporary emulsion? What is a permanent emulsion?

86. Reaction of Fats with Bromine. Dissolve 10 drops of olive oil in a little alcohol, add an equal volume of bromine water and shake thoroughly. Is the bromine absorbed? Explain this reaction.

87. Acrolein Test. Mix a small amount of lard with an equal amount of potassium hydrogen sulphate in a dry dish; by means of a dry stirring rod transfer a small portion of the mixture to a dry test-tube; heat cautiously, and observe the irritating odor of acrolein. Also, moisten a strip of filter paper with a drop of ammoniacal silver nitrate and hold it in the fumes of the acrolein. What happens? Why?

88. Saponification. Place 10 grams of lard in a flask, add 30 c.c. of alcoholic sodium hydroxide solution and warm on a water-bath until the saponification is complete. This point is indicated by the complete solubility of a drop of the solution when allowed to fall into a little water. Now transfer the solution to an evaporating dish containing 40 c.c. of water and heat on a water-bath until all the alcohol has been driven off. Precipitate the fatty acids with hydrochloric acid and cool the solution. Remove the fatty acids which rise to the surface. Reserve the fatty acids for future experiments. Neutralize the remaining solution with a solution of sodium carbonate, and evaporate to dryness. Extract the residue with alcohol, then evaporate this alcohol extract on a water-bath until all the alcohol is removed. This residue is mainly glycerol. Reserve it for use in the following experiments:

89. Reactions of Glycerol. *a. Solubility.* Test the solubility of glycerol in water, ether, and alcohol.

b. Acrolein test. Repeat the test as outlined in Exp. 87, using a few drops of glycerol instead of lard.

c. Borax fusion test. Mix a few drops of glycerol with some powdered borax. Dip a platinum wire in this mixture and fuse it. Note the characteristic blue flame.

d. Dunstan's test. Add phenolphthalein solution to a 5 per cent solution of borax until a permanent pink color is

produced. Add an aqueous solution of glycerol (about 10 per cent), drop by drop, until the color is just discharged. Boil the solution. The color will return if excess of glycerol has not been added.

NOTE.—Any polyhydric alcohol may give this reaction. Ammonia salts also decolorize the solution, but the color does not return on heating.

e. Solution of cupric hydroxide. Add a few drops of sodium hydroxide solution to about 5 c.c. of copper sulphate solution. Cupric hydroxide is precipitated. Add a little glycerol to this suspension and note what occurs.

90. Properties of the Fatty Acids. Prove by the following tests that the precipitate obtained in Exp. 88 consists of fatty acids:

a. Reaction. Dissolve a small portion in ether and add it to about 5. c.c. of alcohol containing 1 drop of phenolphthalein solution and 1 drop of dilute sodium hydroxide solution. Is the color dispelled?

b. Acrolein test. Repeat Exp. 87, using a small portion of the fatty acids instead of the fat. What is your result? Why?

c. Formation of soap. Dissolve some of the fatty acids in sodium hydroxide solution. Is a soap lather produced on shaking some of this mixture with warm water? Add some solid sodium chloride to another portion, to the point of saturation, with continual stirring. Is soap precipitated? Explain this reaction. To a third portion add some calcium chloride solution. Do you get the usual curdy precipitate produced by calcium soaps?

Distinguish between hard soaps and soft soaps.

90a. Preparation of Cholesterol and Lecithin from Egg-yolk.

a. Separate the white of an egg as completely as possible from the yolk. Dehydrate the yolk by one of the following methods:

Method I. Thoroughly mix the yolk with about three times its weight of plaster of paris (50 grams) and let it set overnight. After grinding to a powder it is ready for extraction.

Method II. Place the egg-yolk in a small wide-mouthed bottle or Erlenmeyer flask; add 50 c.c. of acetone and stir it at frequent intervals for an hour. Filter, and then spread the residue out on several thicknesses of filter paper to allow the evaporation of the acetone. The product is now ready for further extraction. This method has the advantage of saving time.

NOTE.—The acetone will extract some of the cholesterol, but, owing to the presence of water, the quantity is small, and for non-quantitative experiments it may be disregarded.

b. Place the product obtained by either of the above methods in a wide-mouthed bottle or Erlenmeyer flask, add 50 c.c. of petroleum ether or ethyl ether (the petroleum ether is the better of the two), and stir at frequent intervals for an hour. Filter. Transfer the residue back to the bottle or to a dry beaker, add 25–50 c.c. more petroleum ether, stir for a minute or two and then filter. Combine the filtrates and evaporate on a steam-bath to about one-quarter the original volume. CAUTION: *Do not heat over a free flame.* Cool, and then add about twice the volume of cold acetone. Crude lecithin will be precipitated. Filter, and preserve both the filtrate and the precipitate. The filtrate contains cholesterol, fat, and other substances, such as lipochromes, etc.

c. Separation of cholesterol from fat, etc. Fats are converted into soap by boiling with alcoholic solutions of sodium or potassium hydroxide. Cholesterol is not changed. Alkali soaps are not soluble in ether, cholesterol is soluble in ether. Hence, we may use the following method: Evaporate the filtrate in *b* to dryness on the steam-bath. Add 25 c.c. of a 10 per cent solution of sodium hydroxide in alcohol. Mix thoroughly, and again evaporate to dryness. Add 25 cc. of ether to the residue, warm, stir thoroughly, and filter. The ether extracts the cholesterol. Evaporate the ether to dryness on the steam-bath and dissolve the residue in about 15 c.c. of hot alcohol. Filter hot on a small filter paper. On cooling the cholesterol will crystallize in

rhombic plates, which often have a reentering (notched) angle. From dry ether, chloroform, and benzene it crystallizes in needles.

90b. Properties of Cholesterol. *a. Microscopic examination.* Mount a few crystals of cholesterol in a drop of water on a slide, and examine them under a microscope. Make a sketch of the crystals.

b. Salkowski's reaction for cholesterol. Introduce a small quantity of cholesterol into a small, clean, dry test-tube and dissolve it in 2–3 c.c. of chloroform; add an equal quantity of concentrated sulphuric acid, while holding the test-tube at an angle of 45°, and shake well by rotating the tube. The upper, or chloroform layer becomes red, and the sulphuric acid layer yellow with a green fluorescence.

c. Liebermann-Burchard reaction for cholesterol. Dissolve a little cholesterol in 2 c.c. of chloroform in a perfectly dry casserole or evaporating dish. Add 10 drops of acetic anhydride, and then 2 drops of concentrated sulphuric acid, and gently shake with rotatory motion. Note the play of colors. It usually becomes first red then purple, then finally a deep blue. Sometimes the final color is a deep bluish-green.

d. Iodine reaction with cholesterol. Place a few crystals of cholesterol on a crucible cover, evaporating dish, or glass slide. Irrigate with a drop of concentrated sulphuric acid, and then a drop of iodine solution. The crystals become first violet, then blue, green and red. Sometimes the final color is black.

All these reactions may be used for the identification of cholesterol.

90c. Properties of Lecithin. To the precipitate obtained in 90*a*, *b*, apply the following tests:

a. Crystallization of lecithin. In a dry test-tube dissolve some crude lecithin in as small a quantity as possible of warm absolute alcohol and place this in a freezing mixture of about −10° C., when the lecithin will crystallize out. It should be filtered cold.

b. Microscopic examination. Mount a little of the lecithin in a drop of cold water and examine with a microscope. Notice the myelin forms.

c. Action of boiling water on lecithin. Warm a little of the crystallized lecithin with water. Note that after a time it turns brown and that the reaction becomes acid.

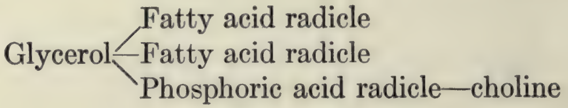
d. Acrolein test. Apply the acrolein test (see Exp. 87) to a small portion of lecithin (either the crude or the purified lecithin may be used). Record your result. What compounds must be present in lecithin if it yields a positive acrolein test?

e. Fusion test for phosphorus. Place a small quantity of lecithin in a porcelain crucible, add 3 or 4 times this amount of a fusion mixture composed of sodium hydroxide and potassium nitrate (5–1) and heat carefully until the resulting mixture is colorless. Cool, and dissolve the mass in a little warm water. Acidify with nitric acid, heat to boiling and add about 5 c.c. of ammonium molybdate solution. If phosphorus is present a yellow precipitate will form. Record your result.

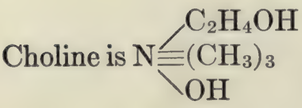
f. Reaction with sodium hydroxide. Boil a small quantity of lecithin with a little sodium hydroxide solution. Notice the fishy odor of trimethylamine that develops. The trimethylamine comes from choline, one of the cleavage products of lecithin.

g. Test for nitrogen. Test another portion of lecithin for nitrogen by Lassaigne's method, Exp. 3, *b*.

From the above, and other more complicated tests, it has been shown that the constitution of lecithin can be represented as follows:



$$\text{Glycerol}\begin{matrix}\diagup\text{Fatty acid radicle}\\ -\text{Fatty acid radicle}\\ \diagdown\text{Phosphoric acid radicle—choline}\end{matrix}$$



$$\text{Choline is } N\begin{matrix}\diagup C_2H_4OH\\ \equiv(CH_3)_3\\ \diagdown OH\end{matrix}$$

90d. Preparation of Cholesterol, Phospholipins (Lecithin and Cephalin), **and Galactolipins** (Phrenosin and Kerasin) **from Brain.**

a. Cut up a sheep's brain in a mortar, add a couple of hornspoonfuls of sand and grind thoroughly. Transfer the macerated brain tissue to a wide-mouthed bottle, add 100 c.c. of acetone and cover with a watch-glass. Stir at frequent intervals for the remainder of the laboratory period and then set aside until the next day. Filter through muslin, and then spread out to dry on several thicknesses of filter paper. After the odor of acetone has practically disappeared from the brain, place it in a mortar, add 50 c.c. of petroleum ether, grind the mixture for a couple of minutes, then transfer it to a wide-mouthed bottle, add another 50 c.c. of petroleum ether, cover, and shake at intervals for an hour. Filter by decantation on to a filter-paper. Shake up the residue with about 25 c.c. more petroleum ether and filter. Combine the filtrates, and label, "Filtrate A." Spread out the residue on several thicknesses of filter paper to allow the petroleum ether to evaporate. After it is dry label it "Residue A." Save both.

b. Isolation of cholesterol from the phospholipins. Evaporate "Filtrate A" on a steam-bath to a small volume (15–20 c.c.). Cool. Add twice the quantity of cold acetone. The phospholipins will be precipitated. Save both the filtrate and the precipitate. Label the filtrate "Filtrate B," and the residue "Precipitate B."

c. Preparation of cholesterol. In the case of brain tissue there is so little simple fat that it is not necessary to treat with alcoholic alkali solution.

Evaporate "Filtrate B" to dryness on the steam-bath, add 20 c.c. of absolute alcohol, heat to boiling, stir, and filter hot on a small dry filter paper. On cooling a good yield of well-shaped cholesterol crystals should be obtained.

d. Properties of cholesterol. Repeat Exp. 90b. Record your results.

e. Properties of the phospholipins. Repeat Exp. 90c,

on the crude phospholipin product labeled " Precipitate B." Record your results.

f. Separation of cephalin from lecithin. Cephalin differs from lecithin in being insoluble in alcohol. Chemically, it differs in that the base united to the phosphoric acid radicle is not choline, but oxyethylamine, $NH_2—CH_2—CH_2OH$.

Treat a portion of " Precipitate B " with alcohol; part will dissolve and part will not. On centrifuging the mixture, a clear alcoholic solution may be separated from the syrupy material that is insoluble in alcohol. The latter is the cephalin fraction and the former is the lecithin fraction.

g. Isolation of the glycolipins. Place the dry " Residue A " in a beaker, add 75 c.c. of alcohol, cover with a watch glass, and boil on a water-bath for one-half hour. Filter the solution *hot* and set away the filtrate for twenty-four hours. Filter and test the precipitate as indicated in the next paragraph.

h. Properties of the glycolipins. 1. Microscopic examination. Mount a small portion of the product in a drop of water on a slide and examine the crystals under the microscope. If you see any, draw them. 2. Solubility. Test the solubility of the crude glycolipins in water, hot and cold alcohol, hot and cold ether, acetone, 10 per cent sodium chloride solution, 0.2 per cent HCl, 0.5 per cent Na_2CO_3 concentrated hydrochloric acid, and 2N NaOH solution. Record your results. 3. Phosphorus. Test for phosphorus according to the directions given in Exp. 90c, (*e*). How does the result compare with lecithin? 4. Test for reducing sugar. Place the remainder of the glycolipins in a casserole, add 20 c.c. of water and 20 c.c. of 2N HCl solution and boil for an hour. Cool, make alkaline with solid sodium hydroxide, filter, and add Fehling's solution. Do you get a reduction?

CHAPTER XII

THE CARBOHYDRATES AND GLUCOSIDES

General Tests on the Carbohydrates

91. Solubility of Typical Carbohydrates. Test the solubility of *arabinose, dextrose, levulose, sucrose, maltose, lactose, starch, dextrin,* and *cellulose* in the so-called "biological solvents": H_2O; 10 per cent NaCl; 0.5 per cent Na_2CO_3; 0.2 per cent HCl; 8 per cent NaOH; concentrated HCl; ether, and alcohol. Use only a pinch of the carbohydrate and about 5 c.c. of the solvent in each case. Record your results.

92. Molisch's Test (Furfuraldehyde Reaction). Place about 5 c.c. of concentrated sulphuric acid in a small test-tube. Incline the tube to an angle of 45° and slowly pour into it about 5 c.c. of a weak solution of any of the carbohydrates mentioned in Exp. 91, to which 2 drops of Molisch's reagent (a 15 per cent alcoholic solution of α-naphthol) has been added. A reddish-violet zone is produced at the point of contact. The reaction is due to the formation of furfuraldehyde by the acid, which then combines with the α-naphthol. At the junction of the two liquids a green ring may at first be formed (because of the slight nitrate or nitrite content of the sulphuric acid), but above this in a short time the reddish-violet will appear. The band disappears after a time. All the carbohydrates and substances containing a carbohydrate group give this reaction.

MONOSACCHARIDES

A. The Pentoses, $C_5H_{10}O_5$.

In the plants and more particularly in certain gums, complex carbohydrates, called pentosans, occur. These

pentosans through hydrolysis by acids may be transformed into pentoses.

93. Action of Strong Hydrochloric Acid. Pentoses on boiling with hydrochloric acid yield furfuraldehyde, which is volatile with steam and may be detected with aniline acetate.

Place about 2 grams of gum arabic in a small flask, add about 25 c.c. 10 per cent HCl solution, and boil. Moisten a piece of filter paper with a drop of anilin acetate (prepared from equal parts of aniline, glacial acetic acid, and water). Hold this paper in the vapor escaping from the flask after most of acid fumes have escaped, a bright crimson color will be formed.

94. Tollen's Phloroglucinol Test. Mix equal parts of concentrated HCl and water, and a little phloroglucinol. Then add a small quantity of pentose solution (a 1 per cent solution of gum arabic may be used), and heat the mixture on a boiling water-bath. The solution gradually becomes cherry red in color, and shows an absorption band between D and E when examined with a spectroscope. Galactose and glycuronic acid will give the same color reaction, but glycuronic acid will not give the same absorption band in the spectroscope, and galactose exhibits no absorption bands.

95. Tollen's Orcinol Test. Repeat Exp. 94, using orcinol instead of phloroglucinol. A succession of colors from red through reddish-green to green is produced. A green precipitate is formed which is soluble in amyl alcohol and has absorption bands between C and D in the spectroscope.

B. The Hexoses, $C_6H_{12}O_6$.

a. Glucose. Synonyms Destrose, grape sugar.

$$(CH_2OH)—(CHOH)_4—(CHO).$$

For the following tests use a 2 per cent solution of glucose.

96. Formation of Esters (Alcohol Reaction). Shake up a little of the glucose solution with a few drops of benzoyl chloride and an excess of sodium hydroxide solution. A precipitate is formed consisting of the benzoyl ester of glucose. Since any alcohol will yield this reaction it would be necessary to determine the melting-point of the ester in order to use this test as a means of identification; however, this may be omitted in this instance.

97. Moore's Test (Aldehyde Reaction). To about 5 c.c. of the glucose solution add an equal volume of sodium hydroxide solution, and boil. The liquid acquires a color ranging from yellow to dark brown. The intensity of the color depends on the quantity of glucose present. The brown color is due to the oxidation of the carbohydrate with formation of alkali salts of certain organic acids (lactic acid, glucic acid, etc.). This test is unreliable as a test for sugar in urine.

98. Action of Concentrated Hydrochloric Acid on Glucose. Boil a few c.c. of the glucose solution with an equal volume of conc. HCl. The solution becomes brown, and black "humic substances" separate out. Levulinic acid is formed and remains in solution. What kind of an acid is levulinic acid?

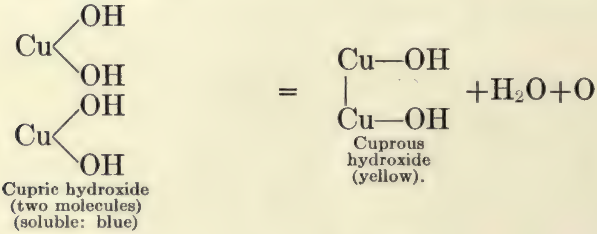
99. Reduction of Metallic Oxides in Alkaline Solutions. To their aldehyde or ketone structure many sugars possess the property of readily reducing the alkaline solutions of the oxides of metals like copper, bismuth and mercury. They also possess the property of reducing ammoniacal silver solutions with the separation of metallic silver. Most of the best-known tests for sugars are based upon this reducing property.

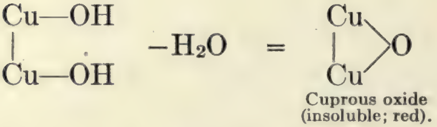
a. Reduction of Fehling's solution. (This solution must be prepared and kept in two separate bottles "A" and "B" and just before using they are mixed in equal proportions. "A" contains 69.28 grams of copper sulphate dissolved in water and made up to a litre. "B" contains 346 grams of Rochelle salt plus 260 grams of sodium hydroxide

dissolved in water and made up to a litre.) The mixed Fehling's solution is of such a strength that 1 c.c. will reduce exactly 0.005 gram of glucose; hence, it may be used for quantitative tests. For qualitative tests it is desirable to dilute the solution before using. Dilute about 5 c.c. of the mixed Fehling's solution with about 10 c.c. of water and heat to boiling; if no precipitate forms (due to the decomposition of the Fehling's solution) add 1 c.c. of the dextrose solution and heat again.

The production of yellow cuprous hydroxide or brownish-red cuprous oxide indicates that reduction has taken place. These changes are indicated as follows:

The alkali converts the copper sulphate into cupric hydroxide, and this, in turn, is reduced by the carbohyhydrate. Equations:





b. Reduction of Fehling-Benedict's sugar reagent. (17.3 grams $CuSO_4$, 173.0 grams sodium citrate and 100.0 grams of sodium carbonate dissolved in a litre of water.) This solution can all be made up in one bottle and does not decompose on standing as does the Fehling's solution, and it is more delicate. Boil a little of this solution in a test-tube and add an equal volume of dilute sugar solution. Compare the result with Fehling's test.

c. Barfoed's sugar solution. (This solution is prepared by dissolving 4.5 grams of copper acetate in 100 c.c. of

water and adding 1.2 c.c. of 50 per cent acetic acid.) Place about 5 c.c. of Barfoed's solution in a test-tube and heat to boiling. Add dextrose solution slowly, a few drops at a time, heating after each addition. If a precipitate does not form upon continued boiling, allow the tube to stand a few minutes and examine. Barfoed's test is specific for monosaccharides.

d. Nylander's reagent (*alkaline bismuth*), (20 grams of bismuth subnitrate and 40 grams of Rochelle salt are dissolved in a litre of 10 per cent sodium hydroxide solution). To about 5 c.c. of the glucose solution add $\frac{1}{2}$ c.c. of Nylander's reagent and heat for several minutes in a boiling water-bath. Note what happens. Explain.

e. Reduction of silver ammonium hydroxide. Place about 5 c.c. of silver nitrate in a perfectly clean test-tube; then add ammonium hydroxide, drop by drop, until the precipitate which first forms just redissolves. Add a few drops of the glucose solution and heat in a boiling water-bath without agitation for a few minutes. A mirror of metallic silver gradually forms.

Write the equations of the above reactions.

100. Phenylhydrazine Reaction (Osazone Test). To about 5 c.c. of the glucose solution in a clean test-tube add about 2 grams of the phenylhydrazine mixture (1 part by weight of phenylhydrazine-hydrochloride and 2 parts by weight of sodium acetate, thoroughly mixed in a mortar), shake well and heat on a boiling water-bath for half an hour, and allow the mixture to cool gradually. Examine the deposit under the microscope and sketch the crystals. Certain other carbohydrates respond to this test but the crystals have different configurations.

Write the reactions (consult your text-book).

101. Fermentation Test. Rub up in an evaporating dish a small piece of yeast ($\frac{1}{4}$ of a yeast cake), with about 25 c.c. of glucose solution. Place the mixture in a saccharimeter and let it stand in a warm place until the next day. Note the result and explain it. What is the gas that

has collected in the upper end of the tube? Prove the presence of alcohol in the liquid by applying the iodoform test. (Exp. 22, *c*.)

102. Determination of the Specific Rotation. The property possessed by all carbohydrates of rotating the plane of polarized light in direct proportion to the amount contained in the solution under examination is utilized for the purpose of determining the percentage of carbohydrate that is present. Some substances, such as glucose, rotate the plane of polarization in the direction of the hands of a watch. These are called dextrorotatory and are designated with a + sign. Substances such as fructose which rotate the plane of polarization in the opposite direction are called lævorotatory and are designated with a − sign.

The "specific rotating power" of a substance is the angle about which the plane of polarized light is turned by a layer 1 cm. thick of a solution containing in 1 c.c. 1 gram of the optically active substance. It is dependent upon the wave length of the light and upon the temperature; hence, it is necessary to specify both of these in stating the value of the specific rotation. With most substances it is impractical to estimate the specific rotation of a 100 per cent solution, but it has been calculated from the rotations of solutions of known strengths. The symbol $(\alpha)_D$ is used to express this value, the *D* standing for sodium light.

The following are the values for a few of the principal carbohydrates in 10 per cent solutions estimated at 20° C.:

Glucose = +52.8°
Galactose = +83.0
Fructose = −93°
Lactose = + 52.5°
Maltose = +138°
Sucrose = + 66.5°
Starch = +199°
Glycogen = +196.6°

The formula is as follows:

$$(\alpha)\frac{20^\circ}{D}=\frac{\alpha\times 100}{C\times l}$$

In which $(\alpha)\frac{20^\circ}{D}$ = specific rotation;

α = observed rotation;
c = concentration;
l = length of tube in decimeters.

The use of the polariscope. The instrument used for the purpose of estimating the amount of an optically active substance is called a polariscope, or polarimeter, and essentially consists of a polarizer and an analyzer, the latter being used to determine the direction and degree of rotation which the light has undergone when passing through the polarizer. The instrument is so arranged that when the analyzer is in one position, one half of the field is fully illuminated while the other half is dark, the two portions being sharply divided by a vertical line. On turning the analyzer to the opposite extreme, the light and the dark portions are transposed. The polariscope is so constructed that a glass tube filled with the liquid to be examined can be placed horizontally in the track of the polarized ray, between the polarizing and the analyzing sections. Before the tube which contains the solution to be analyzed is placed in position, the zero point of the vernier attached to the eyepiece is made to coincide with the zero point of the circular scale of degrees from which the reading is taken. At this point the whole field should be equally illuminated. If it is not, the position of the prisms must be regulated by means of an adjusting screw provided for the purpose, until perfect equality is obtained. The tube is now placed in position and the screw is turned until the two halves of the field are equally illuminated. A reading is then taken by means of the vernier. Read off the number of whole degrees, half degrees, and minutes. Take several readings by moving the lever and coming back to the point where the two halves of the field are equal.

The resulting value gives you alpha. Knowing alpha the concentration of the carbohydrate solution and the

length of the tube, the specific rotatory power may be calculated from the formula given above.

The determination of glucose by means of the polariscope. The amount of glucose in a solution may be calculated from the following equation:

$$X=\frac{\alpha\times 100}{52.8\times 1}$$

The tubes are usually either 100 or 200 mm. in length, but by using a tube of such a length as to render the degrees or arc equal to percentages, the necessity for calculation is obviated. For instance, in estimating glucose, if a tube 189.4 mm. long be used, then the equation becomes

$$X=\alpha$$

and the amount of glucose in 100 c.c. of the solution becomes the observed angle of rotation. If a tube 94.7 mm. in length be used, then the amount of glucose in 100 c.c. is double the observed angle.

There are many types of special polariscopes on the market which are made solely for the purpose of estimating the percentage of sugar in solution. In these instruments the degree of rotation produced by the sugar solution is ascertained by means of a quartz wedge which, when moved by a screw, compensates the rotation; the extent to which the wedge has to be moved to produce compensation is indicated in percentages on the horizontal scale which takes the place of the degrees of arc of the ordinary polariscope. With these special polariscopes, usually called saccharimeters, any bright artificial light may be used, whereas with the other polariscopes, monochromatic (usually sodium) light is used.

b. Fructose. Synonyms: Levulose, fruit sugar.

$$(CH_2OH)—(CHOH)_3—C=O—(CH_2OH).$$

102a. Preparation of Levulose from Inulin. Levulose has been very expensive in recent years. In fact, at times it has been unprocurable. The following method is a little troublesome, but not very expensive.

Inulin may be obtained from dahlia tubers in which it is present in some cases to the extent of 10 to 12 per cent. Inulin on hydrolysis yields levulose.

NOTE.—The dahlia tubers may be obtained from seed growers. Sometimes, imperfect tubers can be obtained very inexpensively.

a. Isolation of inulin from dahlia tubers. (Modification of Dragendorff's Method.) Cleanse fresh dahlia tubers from all adhering dirt, cut them up into pieces, and macerate them in a meat chopper. Add enough water to the crushed tissue to enable it to be stirred with ease, make the mixture alkaline to litmus with $CaCO_3$, boil and filter. If the filtrate is acid add more $CaCO_3$. While the filtrate is still warm, add lead acetate until no further precipitate is formed. Filter. Remove all the lead from the filtrate with H_2S. Filter. Neutralize with ammonia, and evaporate to about one-third the volume. Cool, and to the cold concentrated liquid add about 3 volumes of alcohol. Let stand 24 to 48 hours, and then filter off the crude inulin. To purify; dissolve in water, and boil with animal charcoal, filter, precipitate with 3 volumes of alcohol, filter on hardened filter paper, wash with alcohol and then with ether, and dry over H_2SO_4, or anhydrous calcium chloride. The inulin should now be a pure white powder.

b. Hydrolysis of inulin to levulose. Heat 1 part of inulin with 5 parts of $\frac{1}{2}$ per cent H_2SO_4 for an hour on a water-bath. Add $BaCO_3$ to remove the sulphuric acid, and concentrate to a syrup on a water-bath. Dissolve the syrup in hot alcohol, and then set aside in a cool place. The levulose will crystallize out

103. Properties of Fructose. Repeat Exps. 96 to 102. Do you obtain the same results as with glucose?

NOTE.—(*a*) Although fructose is a ketose, it, nevertheless, reduces metallic oxides in alkaline solution. This is due to the terminal,

$CO—CH_2OH$, group, which is easily oxidizable. Though acetone does not produce metallic oxide, monohydroxy acetone $CH_3—CO—CH_2OH$ does, as it contains the $—CO—CH_2OH$ group.

(*b*) Fructose gives the same osazone as dextrose. Compare the crystals under the microscope. Write the equations to show why the end product in each case is the same.

104. Seliwanoff's Special Test for Fructose. To a mixture of equal parts of concentrated hydrochloric acid and water add a very small quantity of fructose solution and then add a few crystals of resorcinol. Heat. The solution becomes red in color and deposits a brownish-red precipitate which dissolves in alcohol, giving a red solution.

c. Galactose, $CH_2OH(CHOH)_4—CHO$.

Galactose occurs with dextrose as one of the products of hydrolysis of lactose. In general the same reactions are given by galactose as by glucose. If differs from glucose in having a higher dextrorotatory power and in forming a different osazone.

105. Properties of Galactose. Repeat Exps. 94, 99 and 100, using a 1 per cent solution of galactose instead of glucose. Record your results.

106. Mucic Acid Test. Place 50 c.c. of the galactose solution in a beaker; add 15 c.c. of concentrated nitric acid and evaporate the mixture on a boiling water-bath until the volume has been reduced to about 15 c.c. At this point the liquid should be clear, and a fine white precipitate of mucic acid should form.

DISACCHARIDES. $C_{12}H_{22}O_{11}$

a. Sucrose. Synonyms: Saccharose, sugar, cane sugar, and beet sugar.

A 2 per cent aqueous solution of pure crystalline sucrose will be furnished.

107. Properties of Sucrose. Repeat Exps. 96, 97, 98, 99, 101, 102 and 104. Record your results and compare

with the corresponding reactions obtained with glucose and levulose.

108. Hydrolysis of a Disaccharide. To 10 c.c. of the sucrose solution add about 2 c.c. of 10 per cent H_2SO_4 solution and boil for a few minutes. Cool, render alkaline with solid NaOH, and then perform the reduction test. Use the Fehling-Benedict reagent. (Exp. 99, *b*.)

Explain the result after comparing with your result obtained with the reduction test with sucrose in Exp. 107.

b. Maltose, or malt sugar

109. Properties of Maltose. With a 2 per cent aqueous solution of maltose perform Exps. 97, 98, 99, 100, 101, and 104. Compare with the corresponding reactions with glucose, levulose, and sucrose. Make a drawing of the osazone crystals.

c. Lactose, or milk sugar

110. Properties of Lactose. With a 2 per cent solution of lactose perform Exps. 97, 98, 99, 100, 101, 104, and 106, as described under the monosaccharides. Record your results and compare with the corresponding reactions of the preceding carbohydrates.

POLYSACCHARIDES

a. Starch. Synonym: Amylum. $(C_6H_{10}O_5)_n$

111. Preparation of Starch from Potato. Scrape a medium-sized potato as finely as possible with a knife or spatula, collect the scrapings in a casserole, add about 100 c.c. of water, stir thoroughly, and strain through a piece of unbleached muslin. Mix the residue in the beaker with more water, allow the starch to settle, and decant off the supernatant liquid. Repeat this washing and decanting until a pure white residue remains in the bottom of the beaker. Allow the compact mass of starch to drain thoroughly and spread it out to dry. This preparation may be used in the following experiments:

112. Preparation of Starch Paste. Place 200 c.c. of water in a casserole and heat to boiling. In a small beaker mix 2 grams of starch with a few c.c. of cold water and pour the mixture into the boiling water with constant stirring. Boil the starch paste for a few seconds.

Cool, by placing the casserole in a pan of cold water.

113. Action of Alcohol on Starch Paste. Place 10 c.c. of the dilute starch paste in a test-tube and gradually add 95 per cent alcohol until no further action is observed.

114. Action of Iodine on Starch Paste. Place 2–3 c.c. of starch paste in a test-tube and add a drop of iodine solution (1/10 normal iodine solution = 13 grams of iodine dissolved in 30 grams of potassium iodide in 250 c.c. of water and made up to a liter), and observe the production of a deep blue color. Heat the tube and note what happens. Allow the tube to cool and again note what happens. How do you explain these phenomena? Could these color changes be obtained in an alkaline solution? Why?

115. Action of Iodine on Starch Granules. Cut a *very* thin slice of potato, or take a few starch granules, and place on a slide and examine under a microscope. Now put a small drop of iodine solution on the slice of potato and again examine under the microscope. What do you conclude from this experiment in regard to the nature of starch granules.

116. Action of Tannic Acid on Starch Paste. To a small amount of starch paste in a test-tube add an equal volume of a 1 per cent solution of tannic acid. The solution should become strongly opaque and ordinarily a yellowish-white precipitate is formed.

117. Action of Basic Lead Acetate on Starch Paste. To a few c.c. of starch paste add an equal volume of basic lead acetate solution and note what happens.

118. Diffusibility of Starch Paste. Test the diffusibility of starch paste through animal membrane, parchment paper or collodion (see model).

119. Action of Fehling's Solution on Starch Paste. On

mixing equal volumes of starch paste and Fehling's solution and heating to boiling, do you get a reduction?

120. Hydrolysis of Starch. Place about 20 c.c. of starch paste in a small beaker, add 10 drops of concentrated hydrochloric acid, and boil. By means of a small pipette at the end of each minute remove a drop of the solution and place it on a porcelain test tablet, or crucible cover, and make the iodine test (see Exp. 114). As the hydrolysis proceeds, the blue color should gradually fade and finally disappear. At this point, cool a few c.c. rapidly in a test-tube and add an excess of alcohol.

If this solution does not become opaque on addition of alcohol (thereby indicating that all the starch has been hydrolyzed), cool the remainder of the solution and render a portion of it alkaline with solid NaOH and perform the Fehling's test. A positive Fehling's test indicates that a reducing sugar has been formed from the starch. Make a phenylhydrazine test (Exp. 100), on another portion of the hydrolyzed starch. What sugar has been formed?

121. Microscopical Examination of Starch Granules. Examine microscopically and make a sketch of the configuration of the granules of each of the following starches: Potato, corn, arrowroot, wheat, and rice.

b. Dextrin

122. Properties of Dextrin. Repeat Exps. 113, 115, 116, 117, 118, 119 and 120, using a 1 per cent solution of dextrin instead of starch paste. Record your results.

NOTE.—Commercial dextrin usually contains more or less unaltered starch, also more or less reducing sugar. In the former case the iodin may give a blue or violet color (a mixture of blue and red). In the latter case reduction takes place on testing with Fehling's reagent. Pure dextrin gives no coloration with Fehling's reagent.

123. Testing Bread Crust for Dextrin. Test bread crust for dextrin and compare with a portion from the center of the loaf. Record your results.

c. Glycogen

124. Preparation of Glycogen from Scallops. Place a few scallops in a mortar, add a half teaspoonful of sand, and grind the scallops into a pulp, thereby rupturing the cells. Transfer to a casserole, add about 50 c.c. of water, and boil for 20 minutes. Note the opalescence of the solution. At the boiling-point faintly acidify with acetic acid. Why? Filter, and use the solution for the following tests:

125. Iodine Test. To about 5 c.c. of the glycogen add a drop or two of a 10 per cent solution of sodium chloride (the NaCl sol. is not absolutely necessary, but it intensifies the reaction). Then add 2 to 3 drops of iodine solution. Note carefully the color formed. How does it compare with the colors produced by iodine on starch or dextrin?

126. Action of Fehling's Solution on Glycogen. Make a portion of the glycogen solution alkaline. Use solid NaOH. Then perform the Fehling's test. Do you get a positive result?

127. Hydrolysis of Glycogen. Add 10 drops of concentrated hydrochloric acid to 10 c.c. of the glycogen solution, and boil for 10 minutes. Cool. Make alkaline with solid NaOH, and test with Fehling's solution. Now, what is your result?

128. Action of Alcohol, Tannic Acid, and Basic Lead Acetate on Glycogen. Repeat Exps. 113, 116 and 117 on small portions of the glycogen solution, and compare with results obtained on starch and dextrin.

d. Cellulose

129. Solubility of Cellulose. Recall your tests on the solubility of cellulose as noted in Exp. 91. In addition try its solubility in the following "special solvents": *a.* Schweitzer's reagent. (This reagent may be prepared by adding sodium hydroxide solution to a solution of copper sulphate containing some ammonium chloride. A precipitate of copper hydroxide is formed, filtered off, and washed with water.

The moist copper hydroxide is added to concentrated ammonium hydroxide until no more dissolves.) When placed in this solution, filter paper (cellulose) dissolves. On addition of dilute hydrochloric acid, the color is discharged and an amorphous flocculent precipitate of cellulose is deposited.

b. Stannous chloride in aqueous solution, or better, stannous chloride in HCl solution.

c. Mercuric chloride in HCl solution.

130. Formation of Parchment Paper. Into a cold mixture of two volumes of concentrated sulphuric acid and one of water, dip a piece of dry filter paper for a few seconds; remove and wash it at once in water. This treatment converts the filter paper into amyloid (parchment paper), which gives a blue coloration with iodine. Even while wet, parchment paper is very tough as compared with the original dry filter paper.

GLUCOSIDES

Whereas most carbohydrates exist in nature in the free state, there also occur a large number of complex compounds, which on hydrolysis are broken down into a mono- or disaccharide, and one or more other compounds. The hydrolysis is produced both by boiling with acids and by treating with enzymes at body temperature. The sugar is, in most cases, glucose, but certain glucosides yield some other variety of sugar. The substances combined with the sugar may or may not have a physiological action.

131. Reactions of Salicin. *a.* Ascertain if salicin will reduce Fehling's solution. *b.* Hydrolyze about 10 c.c. of a 2 per cent aqueous solution of salicin by adding 15 to 20 drops of dilute sulphuric acid and boiling for about 15 minutes. Now, make alkaline with sodium hydroxide solution and again test for reducing sugar with Fehling's reagent.

132. Tests for Digitalin. The great toxicity of such a glucoside as digitalin makes it advisable to include tests for its detection. Autenrieth advises the following tests:

a. Concentrated sulphuric acid colors pure digitalin orange yellow. This solution soon becomes blood red, changing upon adding a drop of nitric acid or ferric chloride to cherry and blue red.

b. Concentrated hydrochloric acid dissolves it with a golden yellow color, changing with heat to garnet or violet-red.

Only traces of digitalin are necessary for these tests.

133. Scheme for the Identification of the Most Important Carbohydrates. 1. Apply the Molisch test to a few c.c. of the solution. (Exp. 92.) A positive test indicates that some carbohydrate or substance containing a carbohydrate group is present. A negative result by this reaction is very good evidence of the absence of carbohydrates. Consequently, if a negative result is obtained, no further tests need be applied.

2. Test the reaction of the solution to litmus paper. If the solution is alkaline, neutralize or make faintly acid with HCl. Test a few drops of the faintly acid solution with iodine solution. A blue color indicates *starch.* A reddish color indicates *erythrodextrin* or *glycogen.* Solutions of *dextrins* are transparent and the dextrins *are not* precipitated from solution by basic lead acetate. Solutions of *glcyogen* are opalescent and glycogen *is* precipitated from solution by basic lead acetate.

3. Apply Fehling's test (Exp. 99, *a* or *b*) to about 5 c.c. of the original "unknown" solution. Reduction indicates *dextrose, levulose, galactose, pentose, maltose,* or *lactose.*

4. Test another portion of the "unknown" solution with Barfoed's reagent (Exp. 99, *c*). A positive test indicates the presence of *dextrose, levulose, galactose* or *pentose.* A negative Barfoed's test and positive Fehling's test indicates *lactose* or *maltose.*

5. Differentiate between *lactose* and *maltose* by applying the phenylhydrazine reaction (Exp. 100), and the fermentation test (Exp. 101), to small portions of the "unknown" solution. Distinctive osazone crystals are yielded by both

lactose and maltose. Of the two only maltose is fermentable with the yeast ferments.

6. To identify *levulose, dextrose, galactose* and *pentose* proceed as follows: Test by Seliwanoff's reaction (Exp. 104). A positive test indicates levulose. Perform the phenylhydrazine reaction (Exp. 102). Dextrose and levulose yield the same osazone crystals, but dextrose yields a negative Seliwanoff's reaction; hence, a negative Seliwanoff's reaction and positive glucosazone crystals indicate dextrose. Galactose and the pentoses likewise yield distinctive osazone crystals. *Galactose* may also be identified by boiling some of the "unknown" solution with nitric acid. A fine white precipitate of mucic acid is obtained. To further identify the *pentoses* apply the phloroglucinol test (Exp. 94), and the orcinol test (Exp. 95).

7. To identify *sucrose* in the absence of reducing sugars proceed as follows: Add 1 drop of concentrated HCl to about 5 c.c. of the "unknown" solution and boil for one minute. Cool, render alkaline with NaOH solution and test by Fehling's reaction. Reduction indicates the presence of sucrose.

8. *Sucrose* may be detected in the presence of other disaccharides and polysaccharides as follows: Apply Barfoed's test to insure the absence of monosaccharides in the "unknown" solution (levulose is the only one that must be absent); then test by Seliwanoff's reaction. A positive test indicates *sucrose*. Why?

CHAPTER XIII

MONOBASIC UNSATURATED ACIDS, SATURATED DIBASIC ACIDS, AND HYDROXY ACIDS

134. Absorption of Halogens by Unsaturated Acids. Dissolve a few drops of oleic acid in a little carbon tetrachloride, and add to this a few drops of a solution of bromine in carbon tetrachloride. Note what happens. Explain.

135. Elaidic Transformation. Pour about 5 c.c. of oleic acid into a test-tube and add a small piece of sodium nitrite ($\frac{1}{2}$ gram), and two drops of concentrated nitric acid. Allow the mixture to stand a few minutes. Owing to a shifting of the double bonds, the liquid oleic acid is transformed into its solid isomer, *elaidic acid.*

136. Preparation of Oxalic Acid. (Demonstration.) 180 c.c. of concentrated nitric acid are placed in a large flask (2 liters), and the acid is warmed on a water-bath. The flask is then placed in a hood and 50 grams of sucrose added. Torrents of brown fumes are evolved. When this reaction ceases, the liquid is evaporated to about $\frac{1}{4}$ its original volume. On cooling, large, colorless, prismatic crystals of oxalic acid separate.

137. Reactions of Oxalic Acid. *a.* Heat a little oxalic acid on a crucible lid, and note that it volatilizes without charring.

b. Mix about 2 grams of oxalic acid and 2 c.c. of concentrated sulphuric acid in a test-tube containing a cork, a small, bent, glass tube. Note that effervescence ensues, without charring, and that the gas which is evolved may be ignited. What gas is it that is inflammable?

Write the equation of the reaction.

c. Oxidation of oxalic acid with potassium permanganate. Acidify a little oxalic acid solution with sulphuric acid and

warm gently. Add some $KMnO_4$ solution, drop by drop, with constant stirring. It is at first decolorized, but when the oxalic acid is all oxidized the pink color remains. Write the equation of the reaction.

d. Salts of the alkaline earths. To a little dilute ammonium oxalate solution add a few c.c. of $CaCl_2$ or $BaCl_2$ solution. Note the heavy precipitate that is formed in either case. Test the solubility of calcium and barium oxalate in water, in dilute acetic acid and dilute hydrochloric acid.

138. Detection of Lactic Acid ($CH_3CHOH—CO—OH$). *a. Action of very dilute ferric chloride.* To about 5 c.c. of a very dilute solution of ferric chloride (nearly colorless), add a few drops of a dilute solution of lactic acid, or "unknown" solution. The solution becomes deeply yellow.

b. Uffelmann's test. To a 2 per cent aqueous solution of phenol add, drop by drop, an aqueous solution of ferric chloride until an amethyst blue color is obtained. On adding a little dilute lactic acid solution to a few c.c. of Uffelmann's reagent the amethyst-blue color of the reagent is displaced by a straw yellow.

NOTE.—Mineral acids such as hydrochloric acid discharge the blue color, leaving a colorless solution; other organic acids also give a yellow or brownish color. To detect lactic acid in the presence of a mineral acid it is necessary to shake the solution with a few c.c. of ether; to remove the ether by means of a separatory funnel; to evaporate the ether to dryness; to take up the residue in water, and then to perform the test.

139. Reactions of Tartaric Acid.

$$HO—CO—(CHOH)_2—OC—OH.$$

1. Heat a little tartaric acid in a test-tube. Does it char? What does the odor resemble?

2. Are the alkali salts of tartaric acid very soluble? Add a little KNO_3 solution and a few drops of dilute acetic acid to a strong solution of tartaric acid and stir with a glass rod. The acid potassium salt of tartaric acid is precipitated. A similar precipitate is formed when an ammonium salt

is used in place of the potassium salt. Test the solubility of your hydrogen potassium tartrate in water, dilute acetic and dilute hydrochloric acids.

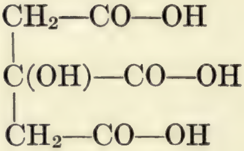
3. Tartaric acid prevents the precipitation of metallic hydroxides by NaOH.

a. To a little ferric chloride solution add NaOH and then some sodium potassium tartrate solution. A reddish-brown precipitate of ferric hydroxide is found, which dissolves on adding the tartaric and gives a yellowish-brown solution.

b. Repeat the same reaction, using $CuSO_4$ solution instead of the ferric chloride. What do you observe? This property is used in the preparation of Fehling's solution.

140. Preparation of Citric Acid from Lemons.

Citric acid.



$$\begin{array}{l} CH_2\text{—}CO\text{—}OH \\ | \\ C(OH)\text{—}CO\text{—}OH \\ | \\ CH_2\text{—}CO\text{—}OH \end{array}$$

Express the juice from half a lemon, add to it an equal volume of water. Filter the mixture and make alkaline with ammonium hydroxide. Add 5 c.c. of a 5 per cent solution of calcium chloride and heat to boiling. What is the formula of the compound formed? Allow the liquid to cool. Does the precipitate remain?

CHAPTER XIV

CARBOCYCLIC COMPOUNDS

A. Benzene and its Homologues

141. Preparation of Benzene, C_6H_6. Mix thoroughly in a mortar 15 grams of benzoic acid and 50 grams of soda lime. Transfer the mixture to a dry round-bottom flask; connect with a condenser, and heat on a wire gauze until no more liquid comes over. To prevent loss by evaporation, surround the receiver with cold water.

Equation:

$$2C_6H_5COOH + Ca(OH) = (C_6H_5-COO)_2 : Ca + H_2O$$

$$(C_6H_5-COO)_2 : Ca + Ca(OH)_2 = 2C_6H_6 + 2CaCO_3.$$

Draw off the oily benzene with a pipette. Shake it with a little dilute sodium hydroxide solution. Recover the benzene. Add to it a little fused calcium chloride. After this mixture has stood for about an hour, decant off the liquid into a large test-tube, connect this tube to a condenser and redistil. Notice the odor of the distillate, which should be preserved for future experiments. A small test-tube fitted with a cork is suitable for this purpose.

142. Reactions of Benzene. *a. Inflammability.* Show that benzene is inflammable by pouring a small quantity of the final distillate obtained in Exp. 141 on a little water in an evaporating dish and igniting it.

b. Action of nitric acid. Treat 2 c.c. of benzene in a flask with a mixture of 4 c.c. of concentrated sulphuric acid and 2 c.c. of concentrated nitric acid. Add the acid mixture, drop by drop, and immerse the flask in cold water after each addition, in order to keep the temperature below 50°. As soon as all the acid has been added, pour the mixture

into water. An oily liquid, nitrobenzene, $C_6H_5—NO_2$, separates and sinks to the bottom. Notice the color and odor of the precipitate. Do not inhale the vapor of this substance. Empty the flask containing it into the jar in the hood.

Write the equation of the reaction.

c. Action of fuming sulphuric acid. Place 5 c.c. of fuming sulphuric acid in a flask, then add to it, drop by drop, 2 c.c. of benzene. Shake the flask, and heat it gently until the benzene, which at first floats upon the surface of the acid, has entirely dissolved. Cool, and divide into two parts. Pour part one into a test-tube two-thirds full of cold water. Does any insoluble substance separate? Pour part two into a small flask containing 10 c.c. of a saturated solution of sodium chloride, and heat this mixture until hydrochloric acid gas is evolved. On cooling, sodium benzene-sulphonate, $C_6H_5—SO_2—ONa$, separates in crystalline plates. Filter on a Büchner funnel; wash the crystals with a little cold water; dry them between filter papers; and preserve them for a later experiment.

d. Apply von Baeyer's test for double bonds to benzene. Agitate about 5 c.c. of benzene with 1 c.c. of 1 per cent solution of potassium permanganate, and 1 c.c. of a 5 per cent solution of sodium carbonate. Does the violet-red color remain unchanged? Compare with the experiments performed on the ethylene and olefine hydrocarbons. (Exp. 15, *c* and *f;* 16, *a.*)

e. Action of bromine on benzene. Place 5 c.c. of benzene and 1 c.c. of a 1 per cent solution of bromine in carbon-tetrachloride in a test-tube and shake for half a minute. Is the bromine dissolved by the benzene? Is the color discharged? Compare with ethylene, amylene and acetylene. Exp. 15, *b* and *e;* and 16, *a.*

143. Oxidation of Side-chains. Mix 2 c.c. of toluene and 5 c.c. of dilute nitric acid in a test-tube and heat. Does any reaction take place? What is the compound formed?

B. PHENOLS

144. Preparation of Phenol, C_6H_5—OH. *a. Phenol from sodium benzene sulphonate.* Weigh the crystals of sodium benzene sulphonate prepared in Exp. 142, *c.* Then fuse double this weight of sodium hydroxide in a porcelain crucible; add the sodium benzene sulphonate crystals, and continue to heat for 3 or 4 minutes. Avoid charring. Cool. Dissolve in a little water and acidify with hydrochloric acid. Then filter. Observe the odor of the filtrate and preserve it for later tests.

b. Phenol from salicylic acid. Mix intimately in a mortar 15 grams of salicylic acid and 50 grams of soda-lime. Transfer the mixture to a dry, round-bottom flask; connect with a condenser and heat on a sand-bath until no more liquid will come over. Collect the distillate in an Erlenmeyer flask surrounded by cold water. Preserve the distillate. Write all equations.

145. Reactions of Phenol. *a. Action of ferric chloride.* Add two drops of ferric chloride solution to 2 c.c. of a 2 per cent aqueous solution of phenol. Note that there is a blue-violet coloration, which disappears on adding mineral acids (HCl).

b. Action of bromine-water. To 5 c.c. of a 2 per cent aqueous solution of phenol add bromine-water until a permanent yellow color is attained. There is first a cloudiness due to the formation of mono- or di-bromophenol. These compounds are characterized by having a very penetrating smell. The further addition of bromine-water produces a precipitate of tribomophenol in the form of yellowish-white needles or flakes.

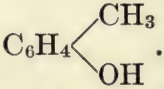
c. Action of Millon's reagent. To a few c.c. of a dilute aqueous solution of phenol add a few drops of Millon's reagent (mercurous and mercuric nitrate) and warm. Note the change in color.

d. Action of nitric acid. Place 10 c.c. of concentrated nitric acid in a porcelain dish and add to it, drop by drop, with constant stirring, about 1 c.c. of an aqueous solution

of phenol. Heat to boiling. On cooling, picric acid, $C_6H_2(NO_2)_3—OH$, is precipitated. Write the equation. Pour the acid mixture into cold water. Examine the crystals that are formed under the microscope. Draw a picture of their configuration.

e. Apply the bromine test to the filtrate obtained in Exp. 144, *a.*

f. Apply reactions *a, b, c,* and *d* to the filtrate obtained in Exp. 144, *b.*

146. Properties of Cresols,  $C_6H_4\begin{cases}CH_3\\OH\end{cases}$. The three cresols, *o*-cresol, *m*-cresol and *p*-cresol, have different physical properties, such as different melting-points, but they have similar chemical properties, and they are extensively sold as a mixture of all three under the name of *tricresol.*

With a 2 per cent solution of tricresol, repeat Exp. 145, *a, b, c* and *d.* Record your results. Do they react similarly to the phenols?

C. Aromatic Amines and their Derivatives

147. Preparation of Aniline from Nitrobenzene. Pour 5 drops of nitrobenzene into a test-tube; add about 1 gram of stannous chloride and 5 c.c. of 8 per cent sodium hydroxide solution. Warm the mixture for a minute or two. The odor of nitrobenzene disappears, giving place to that of aniline. Shake the solution with ether; pour off the ether layer into an evaporating dish; evaporate the ether very carefully by placing the dish on a water-bath containing hot water, but having no flame under it.

148. Bleaching-powder Test for Aniline. Treat the residue obtained in the last experiment with a few c.c. of a clear solution of bleaching powder; a purple-violet color appears which soon becomes dirty red.

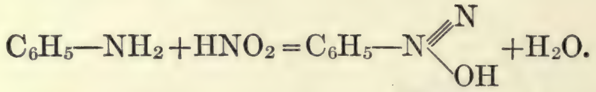
149. Formation of Tribromaniline. To an aqueous solution of aniline or one of its salts, add a little bromine water.

Note the nature of the precipitate.

150. Reaction of Aniline. Shake a few drops of aniline with about 5 c.c. of water; then test its reaction with litmus and with phenolphthalein. What are your results? How does the reaction of aniline compare with reaction of the aliphatic amines? How could you prove aniline to be a primary amine?

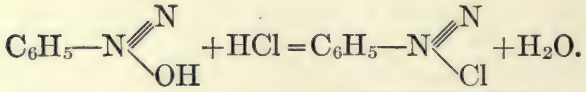
151. Preparation of Acetanilide, C_6H_5—NH—OC—CH_3. Put 3 c.c. of aniline and 5 c.c. of glacial acetic acid in a dry flask and boil under a reflux condenser for an hour and a half. Pour the mixture into water. Acetanilide is precipitated. Recrystallize it from hot water; dry between filter papers; and then determine the melting-point.

152. Action of Nitrous Acid on Aniline. Dissolve 2 c.c. of aniline in 5 c.c. of concentrated hydrochloric acid and 10 c.c. of water. Cool by placing the flask in a pan of ice water. Add 10 c.c. of a cold 10 per cent solution of sodium nitrite. The following reactions take place:



$$C_6H_5{-}NH_2+HNO_2=C_6H_5{-}N\begin{array}{l}\equiv N\\ -OH\end{array}+H_2O.$$

(Diazobenzene)



$$C_6H_5{-}N\begin{array}{l}\equiv N\\ -OH\end{array}+HCl=C_6H_5{-}N\begin{array}{l}\equiv N\\ -Cl\end{array}+H_2O.$$

(Diazobenzene chloride)

Gently heat the solution containing the diazobenzene chloride. A gas is given off. What is it? Note the odor of the solution. What compound is now present in the solution? Write the equation of the reaction that took place on heating the diazonium salt.

153. Preparation of Phenylhydrazine Hydrochloride, C_6H_5NH—NH_2—HCl. Into 100 c.c. of concentrated hydrochloric acid in a beaker, pour slowly, with stirring, 10 c.c. of aniline. Cool the beaker to 0° by placing it in a freezing mixture of one part salt and two parts ice. Add, a few drops at a time, a 20 per cent sodium nitrite solution, until free nitrous acid can be shown to be present. A drop of the

solution diluted with a few drops of water should produce a blue color with starch-iodide paper. Stir constantly while adding the sodium nitrite solution. Dissolve 60 grams of stannous chloride in 50 c.c. of concentrated hydrochloric acid; cool it; and add it slowly to the diazo compound. After half an hour, filter off the phenylhydrazine hydrochloride on a Büchner funnel. Dry between filter papers. Write the equation of the reaction.

154. Properties of Phenylhydrazine. *a. Reduction of Fehling's solution.* Add a small quantity of the phenylhydrazine-hydrochloride to a little mixed Fehling's solution. Does reduction take place?

b. Formation of osazones. Mix a small quantity of the phenylhydrazine-hydrochloride prepared in Exp. 153 with about twice the quantity of sodium acetate, and then add to it a little glucose solution and warm on a water-bath for half an hour. Recall Exp. 100.

D. AROMATIC ACIDS

155. Properties of Benzoic Acid, C_6H_5—CO—OH. *a. Solubility.* Test the solubility of benzoic acid by adding about 0.1 of a gram to 5 c.c. of each of the following solvents: Cold water; hot water; alcchol; ether; dilute sodium hydroxide; and dilute hydrochloric acid. Does the benzoic acid crystallize out from the hot water on cooling? On neutralizing the sodium hydroxide solution with hydrochloric acid, is the benzoic acid precipitated?

b. Sublimation. Heat a little benzoic acid in a casserole. Note the suffocating fumes. Invert a funnel over the casserole. Observe the condensation. Examine the crystals under the microscope.

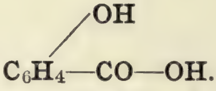
c. Action of nitric acid. To a small quantity of benzoic acid in an evaporating dish add a little nitric acid and evaporate. Observe the odor of the compound that is formed. What is it? Write the equation of the reaction.

d. Action of Millon's reagent. Heat about 0.1 of a gram

of benzoic acid with 2 c.c. of Millon's reagent. Does it yield a color reaction?

e. Action of bromine water. Add about 0.1 of a gram of benzoic acid to a little bromine water. Is the color discharged?

f. Action of ferric chloride. Add about 0.1 of a gram of benzoic acid to a little ferric chloride solution. Is there any change of color?



156. Properties of Salicylic Acid, $\overset{\diagup OH}{C_6H_4}—CO—OH$**.** Repeat the experiments performed on benzoic acid. Make careful note of which tests are positive. Are any of the reactions due to the presence of the —OH radical on the benzene nucleus? Compare Exps. 145 and 146.

E. Aromatic Aldehydes

157. Properties of Benzaldehyde, $C_6H_5—CHO$**.** *a. Production of a silver mirror.* Into a perfectly clean test-tube place 5 c.c. of ammoniacal silver nitrate and add 2 or 3 drops of benzaldehyde. Immerse the tube in a water-bath containing cold water and slowly heat to boiling. Is a silver mirror produced? Compare with the aliphatic aldehydes. (Exp. 35.)

b. Action of Fehling's solution. Shake up a few drops of benzaldehyde with 10 c.c. of water, add 5 c.c. of mixed Fehling's solution and warm. Do you obtain a reduction? Compare with the aliphatic aldehydes. (Exp. 36.)

c. Spontaneous oxidation of benzaldehyde. Spread a few drops of benzaldehyde on a watch-glass and leave exposed to the air for a few days. What change has taken place?

d. Formation of addition products. Shake up 1 c.c. of benzaldehyde with 5 c.c. of a saturated solution of sodium hydrogen sulphite. Collect on a filter paper the crystalline deposit that is formed; wash it twice with a little alcohol; and dry between filter papers. Note that the odor of benzaldehyde has disappeared. Mix some of the crystals with

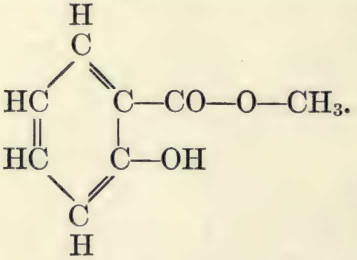
a little sodium carbonate solution and warm. Explain what happens.

e. Action of sodium hydroxide. Mix 1 c.c. of benzaldehyde with 5 c.c. of 8 per cent sodium hydroxide solution and warm. Is aldehyde resin formed? Compare with the results obtained with acetaldehyde. (Exp. 37.)

f. Action of very concentrated alkali. Place in a small flask 10 c.c. of benzaldehyde and 30 c.c. of a 60 per cent solution of potassium hydroxide. Stopper the flask with a cork and shake it until an emulsion is formed, and set it aside until the next day. Add sufficient water to dissolve the crystals of potassium benzoate which have formed. Transfer the mixture to a separatory funnel and extract twice with 25 c.c. portions of ether. Remove the traces of water that remain in the ethereal solution by shaking with anhydrous copper sulphate. Distil off the ether. And finally, distil the benzyl alcohol, $C_6H_5—CH_2OH$, which has formed. It should boil at 206°.

F. POLYSUBSTITUTION PRODUCTS OF BENZENE

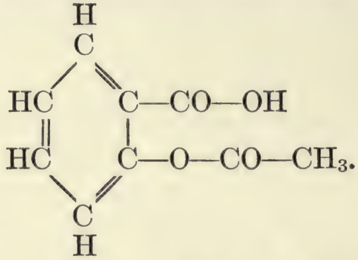
158. Preparation of Methyl Salicylate (Oil of Wintergreen),



Mix together on a watch-glass about 0.1 of a gram of salicylic acid, 5 drops of methyl alcohol, and 5 drops of concentrated sulphuric acid, and gently warm. Observe the odor of the compound formed.

159. Preparation of Aspirin. Heat 5 grams of finely powdered salicylic acid and 4 grams of acetyl chloride under a reflux condenser, on a water-bath. Continue the heating

as long as HCl gas is evolved, then remove the reflux condenser, and boil off the uncombined acetyl chloride. On cooling the aspirin quickly settles out. Test for free salicylic acid by applying the ferric chloride test. (See Exp. 156.)



160. Ionization of Picric Acid in Aqueous Solutions. In a dry test-tube mix about 0.1 of a gram of picric acid and 5 c.c. of petroleum ether and warm on a water-bath. A colorless solution is obtained due to the fact that picric acid does not ionize in this solvent. Add water and mix thoroughly. Ionization results and a yellow color develops. Draw off the aqueous solution and divide it into two parts. To one part add a little sodium hydroxide solution. Note the deepening of the yellow color. What is the product formed?

161 Properties of Gallic Acid, $C_6H_2—(OH)_3—CO—OH$. *a. Reducing action of gallic acid.* Dissolve 0.5 of a gram of gallic acid in 5 c.c. of hot water and add an equal amount of mixed Fehling's solution. Make note of the result.

b. Action of ferric chloride. Dissolve about 0.5 of a gram of gallic acid in 10 c.c. of water and add 1 or 2 drops of ferric chloride solution. Note the color obtained.

c. Formation of ink from gallic acid. Dissolve 0.5 of a gram of gallic acid in 5 c.c. of hot water and add 3 c.c. of a 10 per cent solution of ferrous sulphate and mix by shaking. With a new pen, write on a piece of paper with some of the solution. To about half of the solution add 2 drops of ferric chloride solution and write with this mixture. Compare the results. Put both papers away until the next day and again compare. Explain what took place.

162. Properties of Tannic Acid. A 1 per cent aqueous solution of tannic acid may be used for these tests.

a. Action of ferric chloride. Add a couple of drops of ferric chloride to 5 c.c. of the tannic acid solution. Explain any change that occurs.

b. Reducing action of tannic acid. Test the reducing action of tannic acid with ammoniacal silver nitrate and with Fehling's solution. Record the results.

c. Precipitating action on alkaloids. To a dilute aqueous solution of quinine hydrochloride add an equal quantity of tannic acid solution.

d. Precipitating action on proteins. Dilute 10 c.c. of skimmed milk with an equal volume of water, and add a few drops at a time, a 1 per cent solution of acetic acid. Stir after each addition of acid. Continue to add the acid in this manner until a flocculent precipitate of casein is obtained. Avoid excess of acid. Filter. Add tannic acid to the clear filtrate. Explain the result.

e. Action of tannic acid on gelatin. Add tannic acid solution to a dilute solution of gelatin. Is the gelatin precipitated?

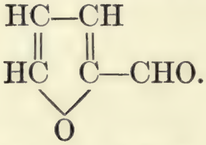
f. Formation of ink from tannic acid. With a little of the tannic acid solution prepare ink according to the technique described for gallic acid. (Exp. 161, *c.*) Do you get a similar result?

CHAPTER XV

HETEROCYCLIC COMPOUNDS

THE term heterocyclic is applied to ring compounds not wholly composed of carbon atoms. One or more links in the closed chain are supplied by other polyvalent elements.

163. Preparation and Properties of Furfuraldehyde. Place



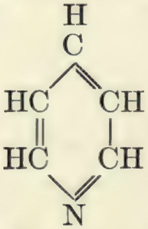
5 grams of bran and 50 c.c. of 2/1 normal hydrochloric acid in a 300- c.c. distilling flask. Connect with a condenser and distil.

a. Observe the characteristic odor of the distillate. The odor of fresh rye bread is due to the presence of furfuraldehyde.

b. To a few c.c. of the distillate add a drop of aniline and a few drops of concentrated hydrochloric acid. Note the intense red color that develops. Compare Exp. 93.

c. Add a few drops of α-naphthol solution to a little of the distillate and float the mixture on 5 c.c. of concentrated sulphuric acid. Note the play of colors at the junction of the two liquids. Compare Exp. 92.

164. Properties of Pyridine. *a. Reaction of Pyridine.* Add



a few drops of pyridine to 5 c.c. of water and test the reaction of the liquid to litmus.

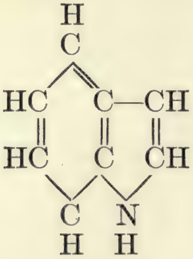
b. Action of ferric chloride. Add a little dilute aqueous solution of pyridine to a solution of ferric chloride. What is the precipitate that forms?

(*c*) *The methyliodide test for pyridine.* Mix 5 drops of pyridine with 5 drops of methyliodide. Heat is evolved, and a solid is formed. (Pyridine methiodide, $C_6H_5N—CH_3I$).

To what class of amines does pyridine belong?

(*d*) *Action of oxidizing agents on pyridine.* Test a small quantity of an aqueous solution of pyridine with Von Baeyer's reagent, concentrated nitric acid, or potassium dichromate and sulphuric acid.

165. Detection of Indole. Indole is one of the products of putrefaction. It may be detected in the products of distillation of putrefied meat as follows:

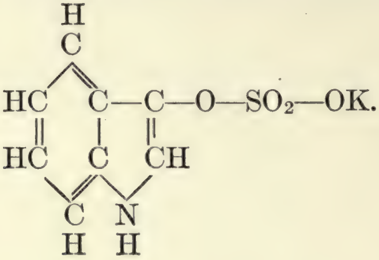


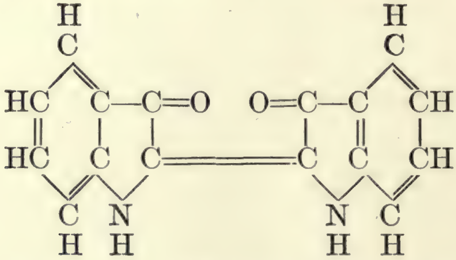
a. Acidify some of the distillate with nitric acid, and add a few drops of sodium nitrite solution. The solution becomes red in color and on standing a red precipitate of nitroso-indole separates out.

b. Formaldehyde reaction (*Konto*). To 1 c.c. of the distillate in a test-tube add 3 drops of formaldehyde solution and 1 c.c. of concentrated sulphuric acid. Shake the mixture and note the appearance of a violet-red color if a trace of indole is present. Repeat with a very dilute aqueous solution of indole (1 : 500,000).

c. Pine wood test for indole. Moisten a pine splinter with concentrated hydrochloric acid and insert it into the solution under examination. The wood becomes cherry-red in color if indole is present.

166. Oxidation of Indican. When indican, which occurs in small quantities in normal urine and in large quantities in certain pathological urines, is treated with acid, it loses its sulphate radical and is converted into indoxyl (hydroxy-indole). If, in addition to the acid, an oxidizing agent is added, the indican will be converted into indigo blue.





a. Place about 10 c.c. of urine in a test-tube. Add an equal volume of HCl, 2 drops of a 1 per cent solution of calcium hypochlorite and 2 to 3 c.c. of carbon tetrachloride or chloroform. Place the thumb over the end of the test-tube and shake the tube thoroughly. The carbon tetrachloride or chloroform will be colored more or less according to the amount of indican present. The color is ordinarily a deep blue, due to the formation of indigo blue; sometimes indigo red is formed instead.

b. Obermeyer's test. To 10 c.c. of urine in a test-tube add 3 to 4 c.c. of carbon tetrachloride and 5 c.c. of Obermeyer's reagent (prepared by adding 2 to 4 grams of ferric chloride [an oxygen carrier] to a liter of concentrated HCl). The result should be the same as in the previous test.

CHAPTER XVI

VEGETABLE ALKALOIDS

THE term *vegetable alkaloid* is generally applied to those basic nitrogenous substances which occur in plants, irrespective of any similarity in properties or constitution. They are characterized by their intense action upon the animal body. Some alkaloids, notably coniine and nicotine, are composed of carbon, hydrogen and nitrogen only, and these are soluble, volatile liquids. Most alkaloids, however, are composed of carbon, hydrogen, nitrogen and oxygen. These are crystalline and non-volatile. They are sparingly soluble in water, but they dissolve in most organic solvents. They combine with acids and form salts, which are soluble in water.

The Detection of Alkaloids

(The following scheme, known as the Stas-Otto process for the detection of alkaloids, was condensed from Autenrieth's book, "Detection of Poisons and Powerful Drugs." Authorized translation by W. H. Warren, Ph.D. (Blakiston)).

167. The Isolation of an Alkaloid from a Cadaver, Stomach Content, etc. Put a portion of the finely chopped material into a large flask, and thoroughly mix with two to three times its weight of absolute alcohol. Add enough tartaric acid solution (10 per cent) to give the mixture a distinct acid reaction after it has been shaken. Usually 20 to 30 drops of tartaric acid is sufficient for a laboratory experiment. Avoid excess. Connect the flask, which should be about half full, with a glass tube (80 to 100 cm. long), which serves as a reflux condenser. Heat on a water-bath with frequent shaking, for about 15 minutes. Cool. Filter.

Wash the residue with more alcohol. Evaporate the filtrate, which must have an acid reaction, to a thin syrup in a small beaker on the water-bath. Then mix with 100 c.c. of water. Filter and again evaporate nearly to dryness. Add a little absolute alcohol, and if a precipitate forms, again filter. Again evaporate the filtered, alcoholic solution on a water-bath, and finally dissolve the residue in 50 c.c. of water. Acidify again with tartaric acid and test small portions of the solution with two or more of the "general alkaloidal reagents." (See below.) If precipitates form, proceed according to directions given in experiment 168, et seq.

General alkaloidal reagents. Most alkaloids yield precipitates with the following reagents:

1. Tannic acid;
2. Picric acid;
3. Mercuric chloride;
4. Iodine in potassium iodide;
5. Potassium mercuric iodide;
6. Phosphomolybdic acid;
7. Phosphotungstic acid, etc.

These reagents, termed the general alkaloidal reagents, also give precipitates with amines, purines and other bases, and with proteins. Consequently, they cannot be considered as specific for the alkaloids, but they are useful in detecting the presence of alkaloids or other nitrogenous basic compounds.

NOTE.—If the unknown substance is a powder, dissolve it in distilled water slightly acidified with tartaric acid; then test small portions of the solution with two or more of the alkaloidal reagents; if precipitates form, proceed as indicated in experiment 168 et seq.

A. EXAMINATION OF THE ETHER EXTRACT OF THE TARTARIC ACID SOLUTION

168. Performing the Ether Acid Extraction. Thoroughly extract the acid aqueous solution (see process of prep-

aration described in Exp. 167), two or three times with ether, using each time about the same quantity of the solvent. Use a separating funnel for this purpose. Pour the combined ether extracts into a dry flask loosely stoppered. Retain the aqueous portion for later tests. Slowly evaporate the ether solution in a small glass upon a water-bath which has been previously heated to 38–40° C. Do not have the gas burning during this operation. Add just a little of the ether at a time to avoid the spreading of the alkaloids.

This residue from the acid ether extract might contain the following alkaloids and coal-tar products:

Picrotoxin
Colchicin
Picric acid

Caffeine
Acetanalide
Phenacetine

Antipyrine
Salicylic acid
Veronal

Examine it only for colchicin, caffeine and picrotoxin.

169. Identification of Colchicin. 1. *Sulphuric nitric acid test.* Concentrated H_2SO_4 dissolves colchicin with a yellow color which a drop of HNO_3 will change to green, blue, violet, wine-red and finally back to yellow.

2. *Nitric acid test.* Concentrated nitric acid dissolves colchicin with a dirty violet color which soon changes, when stirred, to brownish-red and finally to yellow. Addition of dilute NaOH solution, until the reaction is alkaline, produces a beautiful orange-yellow or orange-red color. This is the test by which colchicin can be identified.

170. Identification of Caffeine. 1. *Oxidation test.* Pour a few c.c. of saturated chlorine water over caffeine and evaporate the solution to dryness upon the water-bath. A reddish-brown residue will remain. If a few drops of ammonium hydroxide solution are added, a fine purple red color will immediately appear. This test, known as the murexide reaction, is given by several other purines.

2. *Tannic acid test.* Add a little of the tannic acid reagent to an aqueous solution of caffeine. A heavy white

precipitate appears which is soluble in an excess of the acid. This test is not characteristic of caffeine.

171. Identification of Picrotoxin. 1. *H. Melzer's test.* Put a trace of picrotoxin upon a watch-glass and add 1 or 2 drops of a mixture of benzaldehyde and absolute alcohol Place a drop of concentrated sulphuric acid at the side of the other drops, and tilt the watch-glass so that they will gradually mix. Red streaks will run from the substance through the liquid.

2. *Langley's test.* Mix picrotoxin with three times the quantity of potassium nitrate, and moisten the mixture with 1 *small drop* of concentrated sulphuric acid. Then add strong sodium hydroxide solution in excess and an intense red color will appear.

B. EXAMINATION OF THE ETHER EXTRACT OF THE ALKALINE SOLUTION

(Most of the alkaloids appear here.)

172. Extraction of the Alkaline Solution with Ether. To the aqueous solution obtained in Exp. 167, add enough NaOH solution to make the solution strongly alkaline. The alkali will liberate the alkaloids from their salts and combine with morphine and apomorphine, should they be present. Thoroughly extract this alkaline solution with ether. Place the aqueous solution in a separatory funnel, add an equal volume of ether and shake thoroughly. Repeat this process at least three times. The ether extracts all the alkaloids under these conditions except morphine, apomorphine and narceine. Pour the ether extracts into a dry flask. Retain the aqueous solution.

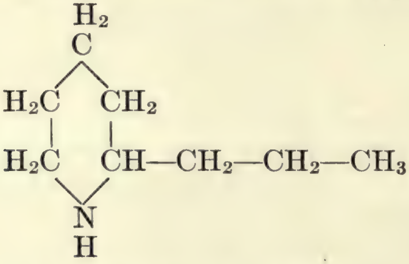
Allow the ether to stand for about an hour; a few drops of water will always separate out. Carefully decant the ether and pour on a small dry dish. The ether should be carefully evaporated, as directed in Exp. 168. The residue, obtained by extracting the alkaline solution with ether, may

contain any alkaloid except morphine, apomorphine or narceine, but especially might it contain the following:

Coniine
Nicotine
Veratrine
Atropine
Codeine
Brucine
Strychnine
Scopolamine
Narcotine
Hydrastine
Pilocarpine
Pyramidone
Quinine
Caffeine
Physostigmine
Cocaine
Antipyrine
Aniline

Examine the solution for the following only: Coniine, atropine, cocaine, codeine, strychnine, brucine and quinine.

173. Identification of Coniine, α-propyl-piperidine.

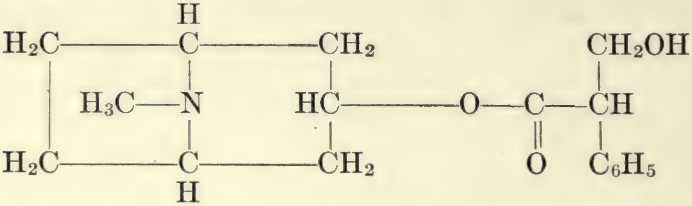


It is a colorless, oily liquid which becomes resinous in contact with air. It possesses an unpleasant, narcotic odor, said to resemble that of mouse urine. Coniine may be recognized by the following tests:

1. *Solubility test.* Dissolve a drop of coniine in just enough cold water to give a clear solution. Gently heat the solution and it will become milky, because coniine is more easily soluble in cold water than it is in hot water. Aqueous coniine solutions have an alkaline reaction.

2. *Crystallization test.* Put a small quantity of coniine upon a watch-glass, or glass slide, and add 1 to 2 drops of HCl. Evaporate to dryness. Coniine hydrochloride will remain. Immediately examine under the microscope. Colorless, or faintly yellow crystals, needle-like or columnar, frequently grouped together in star-shaped clusters, indicate coniine. They show the play of color characteristic of doubly refractive substances.

174. Identification of Atropine, $C_{17}H_{23}NO_3$.



Atropine may be recognized by the following tests:

1. *Vitali's test.* Dissolve the alkaloid in fuming nitric acid, and evaporate to dryness in a porcelain dish upon a water-bath. The residue will be nearly colorless. Moisten the residue when cold with a few drops of a solution of KOH in absolute alcohol. A beautiful violet color will appear and soon a cherry-red. Veratrine behaves in a like manner. Therefore this test is delicate for atropine only in the absence of veratrine.

2. *Odor test.* Heat a small quantity of atropine in a dry test-tube until a white vapor appears. An agreeable odor will be detected at the same time. Then add about 1 c.c. of H_2SO_4, and heat until the acid begins to darken. Dilute at once with about 2 c.c. of water. During the foaming there will be an intense, sweetish odor like that of honey.

175. Identification of Cocaine, $C_{17}K_{21}NO_4$. The structural formula is still a little doubtful. Nearly all the general alkaloidal reagents give precipitates with solutions of cocaine salts. Cocaine may be recognized as follows:

1. *Concentrated acid test.* Concentrated sulphuric and nitric acids dissolve cocaine without color.

2. *Potassium permanganate test.* Add some $KMNO_4$ solution (1 : 100), drop by drop, to a concentrated aqueous solution of a cocaine salt. This reagent will give a violet, crystalline precipitate of cocaine permanganate. In applying the test to the ether residue, dissolve a considerable quantity in 2 drops of dilute HCl and add $KMNO_4$ solution, drop by drop. This is a delicate test for cocaine.

3. *Chromic acid test.* Add a few drops of 5 per cent

chromic acid solution to a solution of cocaine. Each drop will produce a precipitate which will immediately disappear if the solution be shaken. Then add to the clear solution about 1 c.c. of HCl which will produce a more or less crystalline precipitate of orange-yellow cocaine chromate.

176. Identification of Codeine, $C_{17}H_{18}(CH_3)NO_3$. Codeine is the methyl ether of morphine. It crystallizes in colorless, transparent octahedrons, which are often very large.

1. *Sulphuric acid test.* Strong H_2SO_4 dissolves codeine without color. After long contact or upon application of gentle heat, the solution will have a reddish to violet-red color.

2. *Test with Froehde's reagent.* (This is a solution of molybdic acid in 1 c.c. of hot, concentrated, pure H_2SO_4. This solution should be colorless. It does not keep long.) Froehde's reagent dissolves codeine with a yellowish color, which soon changes to green and later to blue. Gentle warming will hasten this change of color.

177. Identification of Strychnine, $C_{21}H_{22}O_2$. Strychnine crystallizes in colorless, shining prisms belonging to the rhombic system.

The sulphur acid dichromate test for strychnine. Dissolve a very small quantity of strychnine in 2 or 3 drops of strong H_2SO_4 upon a watch-glass. The solution should be colorless. Add a fragment of $K_2Cr_2O_7$ and hold it firmly in one place upon the glass; intense blue or blue-violet streaks will come from the potassium dichromate, if the watch-glass be moved up and down. If the entire mixture is stirred the sulphuric acid will be colored a beautiful blue or blue-violet.

178. Identification of Brucine, $C_{23}H_{26}N_2O_4$. Brucine crystallizes from dilute alcohol in colorless, transparent plates

1. *Nitric acid-stannous chloride test.* Strong nitric acid dissolves brucine and its salts with a blood-red color This color, however, is not stable and soon changes to yellowish-red and finally, especially with heat. to yellow. Add a few drops of freshly prepared dilute stannous-chloride solution

to this yellowish-red or yellow solution. An intense violet color will appear.

2. *Nitric acid ammonia test.* Add a few drops of strong HNO_3 to brucine upon a watch-glass and evaporate the solution to dryness. An orange-red to brownish residue will remain. Ammonia will change this color to grass-green. Much strychnine obscures the delicacy of this test.

179. Detection of Strychnine and Brucine when Together. Strong H_2SO_4 dissolves a mixture of these two alkaloids without color. Addition of a trace of nitric acid or a fragment of KNO_3 will produce the red characteristic of brucine. This color will soon change to yellow. Then add a small crystal of $K_2Cr_2O_7$ and stir. The mixture will become blue or reddish-violet if strychnine is present.

180. Identification of Quinine. $C_{20}H_{24}N_2O_2$. 1. *Fluorescence test.* Dissolve the residue from the ether extraction of the alkaline solution in a small quantity of dilute H_2SO_4. This solution will show a blue fluorescence when quinine is present.

2. *Thalleioquine test.* Dissolve quinine in a few drops of very dilute acetic acid, and add 5 to 10 drops of saturated chlorine water. The solution will either be colorless or have a faint blue fluorescence. Addition of NH_4OH in excess will produce an emerald green color.

C. EXTRACTION OF THE AMMONIACAL SOLUTION WITH ETHER AND CHLOROFORM

181. Carefully neutralize the alkaline aqueous solution obtained in Exp. 172 with hydrochloric acid and then make it strongly alkaline with ammonium hydroxide. This ammoniacal solution should then be treated as follows:

a. Extract with ether. The solution should be shaken up with ether in a separatory funnel. On evaporating the residue from this ether extract one will obtain any apomorphine that may be present. Traces of morphine will also appear in this residue.

b. Extract with chloroform. This extract will contain morphine and narceine should they be present.

182. Identification of Apomorphine, $C_{17}H_{17}NO_2$. Apomorphine is freely soluble in alcohol, ether, amyl alcohol and chloroform.

Apomorphine may be recognized by the following tests:

1. *Husemann's test.* Dissolve apomorphine in a strong H_2SO_4 solution and add a drop of strong HNO_3. An evanescent violet will appear and soon change to blood-red and later to yellowish-red.

2. *Pellagri's test.* Dissolve apomorphine in dilute HCl or H_2SO_4 and then neutralize with $NaHCO_3$. Then add 1 to 4 drops of an alcoholic solution of iodine and shake thoroughly for several minutes. The solution will have a blue-green color. Extract with ether and this solvent will have a violet color.

3. *Test with Froehde's reagent* (*see Exp.* 176, 2). This reagent dissolves pure apomorphine with a green color. When the alkaloid has been acted upon by air to any extent, the color will be violet.

183. Identification of Morphine, $C_{17}H_{19}NO_3$. 1. *Sulphuric acid-iodic acid chloroform test.* To a portion of ammoniacal solution add a little dilute H_2SO_4, then a few c.c. of iodic acid solution and finally a small quantity of chloroform. Shake the mixture vigorously. A violet color in the chloroform caused by free iodine indicates morphine.

2. *Nitric acid test.* Strong HNO_3 dissolves morphine with a blood-red color which gradually changes to yellow. Stannous chloride solution does not produce a violet color in this solution. (Distinction from brucine.)

3. *Ferric chloride test.* Add 1 to 2 drops of *neutral* ferric chloride solution to a neutral solution of a morphine salt. A beautiful blue color will appear.

4. *Silver test.* Warm a solution of a morphine salt with $AgNO_3$ solution and an excess of NH_4OH. Morphine will produce a gray precipitate of metallic silver.

CHAPTER XVII

PROTEINS

184. Detection of the Elements Contained in Proteins. *a. Detection of carbon and hydrogen.* Take a small quantity of dry protein (casein or egg-albumin) and test for carbon and hydrogen according to the technique outlined in Exp. 1.

b. Detection of nitrogen and sulphur. Heat a few c.c. of egg-albumin solution with an equal volume of strong sodium hydroxide solution. Hold a piece of moist, red litmus paper in the fumes. Explain.

Dilute this solution with about two volumes of water, and add several drops of lead acetate solution. Explain.

c. Detection of phosphorus. Place about $\frac{1}{4}$ gram of casein and 5 c.c. of concentrated nitric acid in an evaporating dish and boil until nearly dry. Add 10 to 15 c.c. of water and a little ammonium molybdate solution.

d. Detection of iron. Mix a small quantity of dried blood in a crucible with three to four times its quantity of fusion mixture and heat until all the organic matter has been oxidized. Cool. Dissolve the residue in dilute HCl; filter; and test the filtrate for ferric iron by adding potassium ferrocyanide.

Does iron occur in many proteins?

NOTE.—Nitrogen, sulphur and phosphorus may also be detected after fusion with sodium. See Exp. 2, 4 and 5.

185. Preparation of Egg-albumin Solution. Carefully segregate the white of an egg from the yolk. Place the white in an evaporating dish and cut it with a pair of scissors; add a little cold water and continue the cutting with the scissors. Continue to add water until about ten times the volume of the egg-white has been added. Filter first through muslin and then through filter paper.

A nearly colorless solution containing about 1 per cent of protein is obtained by this process.

PRECIPITATION REACTIONS OF PROTEINS

186. Influence of Strong Mineral Acids. *a.* Put about 5 c.c. of strong nitric acid in a small test-tube. Hold the tube at an angle of 45° and by means of a pipette allow a few c.c. of egg albumin solution to flow slowly down the side of the tube. Note the precipitate which is formed at the junction of the two liquids. This is known as Heller's ring test for proteins.

Repeat, using strong hydrochloric acid, also sulphuric acid.

Record your results.

187. Influence of Strong Organic Acids. Add a few drops of glacial acetic acid to a little egg albumin solution. Is the protein precipitated?

188. Precipitation by Salts of the Heavy Metals. *a. Mercuric chloride.* Add 1 to 2 drops of mercuric chloride solution to a little egg albumin solution. Note what happens. Add some saturated sodium chloride solution. Does any change take place? Now add a few drops of dilute hydrochloric acid. Again note what happens.

Record your results.

b. Copper sulphate. Add copper sulphate solution, drop by drop, to a little egg albumin solution. Note the color of the solution formed. Add some sodium hydroxide solution. Does the color change?

To a little egg albumin solution add some Fehling's solution. A violet color is produced. How, then, could one detect a reducing sugar in a solution also containing albumin?

c. Lead acetate. Add a few drops of lead acetate to some egg albumin solution. Note the nature of the precipitate. Egg albumin solution or milk is used as an antidote for lead or mercury poisoning. Why?

d. Ferric chloride. To a little egg albumin solution in a test-tube add ferric chloride solution, drop by drop, until

an excess of the reagent has been added, noting any changes which may occur.

189. Precipitation by Alkaloidal Reagents in Acid Solutions. *a. Potassium mercuric iodide.* Add to 3 or 4 c.c. of egg albumin solution 1 drop of dilute hydrochloric acid and 2 drops of the potassium mercuric iodide solution.

b. Phosphotungstic acid. Slightly acidify a solution of egg albumin with either hydrochloric acid or sulphuric acid and then add a little phosphotungstic acid reagent.

c. Phospho-molybdic acid. Follow the same technique outlined in *b,* using phospho-molybdic acid instead.

d. Hydroferrocyanic acid. To 5 c.c. of egg albumin solution in a test-tube add a few drops of acetic acid. Shake; and then add, drop by drop, potassium ferrocyanide solution.

This test is often used clinically for detecting protein in urine. The test is not so delicate in the presence of netural salts.

e. Trichloracetic acid. To a little egg albumin solution in a test-tube add an equal volume of a 10 per cent solution of trichloracetic acid.

f. Picric acid. Add picric acid, drop by drop, to a little egg albumin solution, which has previously been made acid with very dilute acetic acid.

Record your results in each of the above tests.

190. Action of Alcohol. *a.* Put 3 c.c. of egg albumin solution in a test-tube and add 10 c.c. of alcohol. Remove a small portion of the precipitate and test its solubility in water. Allow the remainder of the precipitate to remain in contact with the alcohol until the next laboratory session. At that time again test its solubility in water. Do you obtain the same results? Explain.

b. To 5 c.c. of alcohol add 1 drop of 0.2 per cent HCl, then add 1 c.c. of egg albumin solution.

c. To 5 c.c. of alcohol add 1 drop of dilute NaOH, then add 1 c.c. of egg albumin solution.

If any precipitate forms in *b* or *c,* test its solubility in water as in *a.* Compare your results.

191. Coagulation by Heat. *a.* Heat 5 c.c. of egg albumin solution in a test-tube to boiling. Note any change in the appearance of the solution. Is there a definite coagulum?

b. To 5 c.c. of egg albumin solution in a test-tube add 2 drops of dilute acetic acid. Then heat to boiling. Do you get a coagulum?

c. Repeat *b*, using 2 drops of glacial acetic acid instead of the dilute. Do you get the same results as in the previous test?

d. Heat 5 c.c. of egg albumin solution after adding 3 drops of 10 per cent $NaCO_3$. Do you get a coagulum?

192. Coagulation Temperature. Add 2 drops of dilute acetic acid to 5 c.c. of egg albumin solution in a test-tube. Put a thermometer in the solution and place the test-tube in a beaker of water. Heat the water and determine carefully the temperature at which coagulation occurs. Both the water in the beaker and the solution in the tube should be gently stirred from time to time. The solution becomes cloudy just before it coagulates.

193. Color Reactions of Proteins. *a. Biuret reaction.* Mix 2 to 3 c.c. of the egg albumin solution with 5 c.c. of 8 per cent sodium hydroxide solution and 2 drops of a 0.5 per cent solution of copper sulphate. *Caution.* Always use the same quantity of the alkali and of the copper sulphate, as variations in the amounts of the copper sulphate will lead to errors in your conclusions.

Gies' biuret reagent. Gies has recently devised a reagent which overcomes the above-mentioned difficulties. This reagent consists of 8 per cent NaOH solution, to which enough dilute copper sulphate solution has been added to impart a slight blue color to the liquid. The copper sulphate must be added a drop or two at a time, with thorough shaking after each addition. When using this reagent proceed as follows: Take two clean test-tubes and put 2 to 3 c.c. of the reagent in each. To one test-tube add an equal volume of the egg albumin solution and to the other add

an equal volume of water. The tube containing the protein should have a distinct violet or pink color, whereas the solution in the other (control) tube is pale blue.

This reaction is due to the presence of at least two —CO—NH— groups. (See Exp. 78, *d,* and 188, *b.*)

b. Millon's reaction. To a little of the egg albumin solution add a few drops of Millon's reagent (solution of mercurous and mercuric nitrates). A precipitate forms, which, on heating, becomes brick-red. The red color constitutes the essential part of the test.

On what group in the protein molecule does this test depend? See Exp. 145, *c,* and 156.

Repeat this test after adding some sodium chloride to the egg albumin. What takes place? Explain.

c. Xanthoproteic reaction. To 4 to 5 c.c. of the egg albumin solution add 2 c.c. of concentrated nitric acid. A white precipitate is produced (this white precipitate is not produced with all proteins or if the protein solution is too weak). Boil. The precipitate or liquid turns yellow. Cool, and render distinctly alkaline with ammonium hydroxide. An orange color is produced.

On what group in the protein molecule does this test depend? See Exp. 145, *d.*

d. Hopkins-Cole reaction. Put 2 to 3 c.c. of egg albumin solution and an equal volume of Hopkins-Cole reagent (an aqueous solution of glyoxylic acid), in a test-tube and mix thoroughly. In a second tube put 5 c.c. of concentrated sulphuric acid. Hold this tube at an angle of 45° and by means of a pipette allow the albumin-glyoxylic acid mixture to run slowly down the side. A reddish-violet color forms at the junction of the two liquids. This color is due to the presence of the *tryptophane group* in the protein molecule.

e. Apply the Molisch test (Exp. 92), to a few c.c. of concentrated egg-white solution. If no color is produced, set the tube aside for a few minutes and again examine. If a positive test is obtained, what group must be present in the molecule?

194. Separation of Proteins by Means of Neutral Salts. For these experiments 1 part egg white is dissolved in 10 parts of 1 per cent NaCl.

a. Separation of a globulin from an albumin by means of sodium chloride. Add to 10 c.c. of egg-white solution in a small beaker an equal volume of a saturated solution of sodium chloride. Do you obtain a precipitate? Add finely powdered sodium chloride to this mixture until the solution is saturated and a slight residue of sodium chloride remains (this will require 3.6 grams of the solid). The globulin is precipitated. Filter the solution; add to the filtrate 1 drop of 2 per cent acetic acid and heat to boiling. A coagulum indicates albumin.

b. Separation of a globulin from an albumin by means of magnesium sulphate. Follow the same technique outlined in *a*, using $MgSO_4$ instead of NaCl. Do you get the same results?

c. Separation of a globulin from an albumin by means of ammonium sulphate. Add to 10 c.c. of egg-white solution an equal volume of a saturated ammonium sulphate solution. The globulin is precipitated. Filter, and divide the filtrate into two parts. To part one add 1 drop of a 2 per cent acetic acid solution and boil. A coagulum indicates albumin. To part two of the filtrate, add solid ammonium sulphate to point of saturation. (Since this solution is half saturated, about 4 grams will be required to effect saturation of 10 c.c.) The albumin is precipitated. Filter, and test the filtrate for protein with biuret reagent.

195. Preparation and Properties of Alkali Metaprotein. Add to 10 c.c. of egg-white solution 1 c.c. of 10 per cent sodium hydroxide solution. Place the test-tube in a water-bath at 50° C. and allow to stand for 30 minutes; the protein is partly converted into alkali-metaprotein. Heat a small portion of the solution to boiling. Does it coagulate? Neutralize another portion with 0.2 per cent HCl. The metaprotein is precipitated. Avoid excess of acid. Put a portion of the precipitate in distilled water. Is it

soluble? Test the solubility of another portion in 2 per cent acetic acid.

196. Preparation and Properties of Acid-metaprotein. Dissolve 1 gram of dried egg-white in 10 c.c. of 0.4 per cent HCl and keep in an incubator at 38° C. for 24 hours. If the solution is not perfectly clear, filter. Heat a small portion of the solution to boiling. Does it coagulate? Carefully neutralize the remainder of the solution with 0.5 per cent $NaCO_3$ solution. Then test the precipitated acid-metaprotein as follows: *a.* Solubility in distilled water; *b.* Millon's reaction; *c* Hopkins-Cole reaction; *d.* Biuret reaction.

197. Separation of the Products of Acid Hydrolysis of Proteins. This separation scheme may be applied to a portion of the above acid metaprotein solution or to a filtered solution of Witte's peptone (5 per cent).

Carefully neutralize. If a precipitate appears, acid metaprotein is present. Filter, and heat the filtrate to boiling. If a precipitate appears, unaltered coagulable protein is present.

Filter, if necessary, and half saturate with ammonium sulphate. (Add an equal volume of saturated ammonium sulphate solution.) If a precipitate appears, primary proteose is present.

Filter; saturate the filtrate with solid ammonium sulphate and heat to boiling.

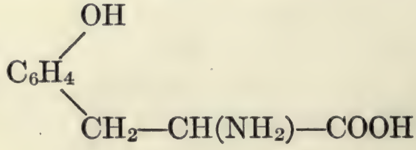
If a precipitate appears at this point secondary proteoses are present.

Filter, if necessary, and apply the biuret and xanthoproteic tests to the filtrate.

If the tests are positive, peptones are present.

198. Isolation of a Typical Amino-acid from a Protein.

Tyrosin



$$C_6H_4\begin{cases} OH \\ CH_2—CH(NH_2)—COOH \end{cases}$$

from casein.

Dissolve 100 grams of casein in 1 liter of 0.1 normal Na_2CO_3, add 1 gram of trypsin, 5 c.c. of toluene (to prevent putrefaction), shake well, and keep in a constant temperature bath at 37° C. for 7 or 8 days. The solution gradually becomes cloudy and tyrosin settles out. Filter. Dissolve the precipitate in 0.1 normal HCl, decolorize by boiling with finely pulverized animal charcoal and filter. Cool and carefully neutralize with ammonia. Crystals of fairly pure tyrosin may be obtained by this method.

199. Properties of Tyrosin. Make the following tests on the tyrosin crystals obtained in the last experiment:

a. Microscopical examination. Place a minute crystal of tyrosin on a slide, add a drop of 0.1 normal NH_4OH, and as soon as the tyrosin has dissolved allow the ammonia to evaporate by holding the slide above a Bunsen flame. The tyrosin crystallizes in characteristic bunches of fine needles. Make a drawing of those you obtain.

b. Solubility. Test the solubility of very small amounts of tyrosin in cold and hot water, cold and hot 95 per cent alcohol, dilute NH_4OH, dilute NaOH and dilute HCl.

c. Color tests. Dissolve a small amount of the tyrosin crystals in a few c.c. of water and then perform the protein color tests, i.e., repeat Exp. 193 with this solution. Make careful notes of which ones are positive.

d. Mörner's test. Add about 2 c.c. of Mörner's reagent (1 volume formalin, 45 volumes water, and 55 volumes concentrated H_2SO_4), to a little of the tyrosin in a test-tube, and *gradually* raise the temperature to the boiling-point. A green color results.

e. Reaction with copper carbonate. Add a little copper carbonate to a few c.c. of the tyrosin solution and boil. Do you get the blue color obtained with aliphatic amines? (See Exp. 74, *d.*)

200. Preparation of a Crystalline Protein. *Edestin from hemp-seed.* Carefully grind about 25 grams of hemp-seed in a mortar. Put only a small amount of the seed in the mortar at a time and add a few grains of sand with each

addition of the seed. Transfer the crushed seed to a casserole containing about 200 c.c. of 5 per cent NaCl solution, which has been heated to *exactly 60° C.* Keep the temperature at 58-60° for one-half hour. This can be accomplished by keeping a very low flame under the casserole and stirring occasionally with the thermometer. If the temperature rises above 60°, remove the flame. Filter hot into a *warm beaker* and through a *warm funnel* containing a fluted filter paper. The beaker and funnel should be warmed as follows: In another beaker heat about 100 c.c. of 5 per cent NaCl to about 70°; pour this through the funnel into the beaker that will be used to collect the edestin solution; then pour it back into the first beaker. The supernatant liquid of the saline extract of hemp-seed should now be transferred to the warm funnel and the funnel should be covered with a watch-glass to prevent loss in heat from the solution. Finally transfer all the solid mass to the funnel and wash it with the saline solution that was used to warm the beaker and funnel. At the time of washing, this solution should be just 60°. Set the filtrate aside to cool slowly.

At the next laboratory period, decant off most of the liquid carefully and filter off the solid. Retain the precipitate and the filtrate.

201. Properties of Edestin. *a. Microscopical examination.* Place a very small amount of the precipitate obtained in the last experiment on a glass slide; cover; and examine under a microscope. Make a sketch of the crystals.

b. Solubility. Test the solubility of edestin in the "biological solvents" (see Exp. 91). Record results.

c. Protein color tests. Apply the following tests to the edestin crystals: Xanthoproteic, Millon's biuret, and Hopkins-Cole. Also test for sulphur. Record your results.

d. Coagulation test. Try the coagulability of edestin in a neutral solution by placing a small amount of the crystals in a test-tube and heating to boiling. Now add a few drops of dilute HCl. Is there any change?

Apply the following tests to the filtrate obtained in Exp. 200.

e. Influence of protein precipitants. Test with nitric acid, trichloracetic acid, tannic acid, picric acid, lead acetate, etc.

f. Saturation with sodium chloride. Saturate some of the filtrate with solid NaCl. What happens? How does this result compare with that obtained upon saturating albumin solutions?

g. Effect of dilution. Place some of the filtrate in a large beaker and add 10 or 15 volumes of water. What happens? From the foregoing tests, what kind of a protein would you judge edestin to be?

202. Preparation of a Prolamine. (Gliadin from wheat.) To 50 grams of wheat flour gradually add enough water to make a stiff dough (about 25 c.c.). Knead the dough with your hands for 2 minutes, then let it stand for half an hour. At this time knead the dough in a piece of cheesecloth under a stream of cold water until all the starch is washed out. The sticky mass that is left is a mixture of gliadin and glutenin known as *gluten.* Cut the gluten into small pieces with a knife or scissors. Transfer the pieces to a small flask containing 100 c.c. of 70 per cent alcohol. Extract the gliadin by boiling the mixture on the steam-bath for 30 minutes. Filter hot. Repeat the extraction process with another 100 c.c. of 70 per cent alcohol. Again filter hot. Combine the filtrates and evaporate to about one-fourth of the original volume. Cool and then add 10 c.c. of 2/1 normal NaCl solution little by little with constant stirring. Allow the precipitate to settle out, filter off and dehydrate with cold 95 per cent alcohol. Spread out on a watch-glass and dry in a desiccator.

203. Properties of Gliadin. Make the following tests on the gliadin obtained in the last experiment.

a. Solubility. Test the solubility of very small amounts of gliadin in 5 c.c. portions of the "biological solvents" (Exp. 91), and in hot and cold absolute alcohol. Record your results.

b. Protein color tests. Dissolve the remainder of your gliadin preparation in 0.1 normal HCl and then perform the protein color tests (Exp. 178). Record your observations.

204. Properties of Gelatin. Gelatin, although often classed with the albuminoids (a class of proteins that are characterized being soluble in all neutral solvents), is in reality a derived protein. It is formed by partial hydrolysis of a typical albuminoid, *collagen*, which is found in tendon, cartilage and bone.

a. Solubility of gelatin. Cut a piece of sheet-gelatin into small pieces (1 cm. square). Place one piece in 5 c.c. portions of each of the "biological solvents" (Exp. 91), and let the tubes stand for 5 minutes. Note any change in the appearance of the gelatin in the tubes. In another tube place two pieces of gelatin and 5 c.c. of water; heat gently by placing the tube in hot water. In the hot water the gelatin dissolves. Cool. It will gel. This is the best test for gelatin. (Solutions of less than 1 per cent concentration do not gel readily.)

For the following tests a 1 per cent solution of gelatin is supplied.

b. Protein color tests. Repeat Exp. 193 with portions of the gelatin solution. Some of the color tests are positive with gelatin, others are not. Make a record of your results.

c. Test for sulphur. Repeat Exp. 184, *b*. Is sulphur present? From the results you obtained in this test and in the color tests, what do you conclude in regard to the composition of gelatin?

d. Precipitation test. Is gelatin precipitated by half saturating the solution with ammonium sulphate (use 5 c.c. of the gelatin solution and 5 c.c. of saturated ammonium sulphate)? By 95 per cent alcohol? By lead acetate? By picric acid? By tannic acid?

e. Effect of boiling in acid solution. Dissolve 1 gram of gelatin in 10 c.c. of warm water; add 1 c.c. of 2/1 normal HCl and boil for 5 minutes. Cool the tube under the tap.

If it does not gel, neutralize with 2/1 normal NaOH solution, and observe if it gels. If the gelatin should gel at this point, reacidify and heat again for 2 minutes; cool; neutralize and test its ability to gel. Would excessively acid fruit and too long boiling interfere with the making of jellies?

205. Solubility of the Albuminoids (Scleroproteins). Test the solubility of hair, finger-nails and horn shavings in the "biological solvents" (Exp. 91). Record your results.

CHAPTER XVIII

THE COLLOIDAL STATE OF MATTER

A. Introduction

206. Crystalloids and Colloids. In an article published in Philosophical Transactions in 1861, an English chemist, Thomas Graham, differentiated all substances into two classes; those which rapidly diffuse through organic membranes (vegetable parchment paper and fish bladders, etc.) and those which either do not diffuse through such membranes, or diffuse very slowly through them. The substances belonging to the first class readily form crystals from saturated solutions. They are such compounds as salt, sugar, urea, etc. Those belonging to the second class are not generally found in the crystalline condition. They are such compounds as silicic acid, soluble alumina, and certain organic compounds, viz., gum arabic, caramel, starch, dextrin, tannin, albumin, etc. Graham called the former class *crystalloids* and the later *colloids*.

207. Graham's Views on Colloids. Graham used diffusion for separating the one class from the other, and he termed this separation *dialysis* and the apparatus used in effecting such separations a dialyzer. When a mixture of the two classes is placed in a dialyzer the crystalloids pass through and the colloids remain behind. The results, however, are not quantitative.

Some of the conclusions that Graham adduced from his studies on diffusion may be obtained from the following quotations from his writings:

" I may be allowed to advert again to the radical distinction assumed in this paper to exist between colloids and crystalloids in their intimate molceular constitution.

Every physical and chemical property is characteristically modified in each class. They appear like different worlds of matter, and give occasion to a corresponding division of chemical science. The distinction between these kind of matter is that subsisting between the material of a mineral and the material of an organized mass."

Again, " The phenomena of the solution of a salt or crystalloid probably all appear in the solution of a colloid, but greatly reduced in degree. The process becomes slow; time, indeed, appearing essential to the colloidal changes. The change of temperature, usually occurring in the act of solution, becomes barely perceptible." . . . " The colloid, although often dissolved in large proportion by its solvent, is held in solution by a singularly feeble force. Hence colloids are generally displaced and precipitated by the addition to their solution of any substance from the other class. Of all the properties of liquid colloids, their slow diffusion in water, and their arrest by colloidal septa, are the most serviceable in distinguishing them from crystalloids. Colloids have feeble chemical reaction." . . . " It is difficult to avoid associating the inertness of colloids with their high equivalents, particularly where the high number appears to be attained by the repetition of a smaller number. The inquiry suggests itself whether the colloid molecule may not be constituted by the grouping together of a number of smaller crystalloid molecules, and whether the basis of colloidality may not really be this composite character of the molecule."

This paper concludes with a discussion of " Osmose," and the views expounded are essentially those that we hold to-day. " It now appears to me that the water movement in osmose is an affair of hydration and of dehydration in the substance of the membrane or other colloid septum. The outer surface of the membrane being in contact with pure water tends to hydrate itself to a higher degree than the inner surface does, the latter surface being supposed to be in contact with a saline solution." Where the membrane

comes in contact with the solution, " the degree of hydration is lowered, and the water must be given up by the inner layer of the membrane and it forms the osmose."

This is essentially the view held to-day in regard to the passage of water through semi-permeable membranes in the measurement of osmotic pressure.

Graham called the colloidal solutions obtained with water as the solvent " hydrosols," or simply " sols," and those which formed a gelatinous mass in water " hydrogels," or " gels." If alcohol was used as the solvent, he called the product " alcosols " and " alcogels," etc.

As our knowledge of colloids has increased, it has been shown that it is incorrect to speak of colloidal substances as a particular class, as Graham did. Certain substances may form a colloidal solution with one solvent, and dissolve as crystalloids in another. E.g., soaps dissolve in alcohol as crystalloids, but in water they are colloids. In fact, by proper manipulation almost any solid can be produced either as a crystalloid or as a colloid. Many proteins have been crystallized and sodium chloride has been obtained in colloidal solution.

Consequently, it is not strictly correct to speak of a substance as a *colloid*, it should be designated as being in the *colloidal state*.

B. Preparation of Colloidal Solutions

A fairly large number of methods have been devised for preparing colloidal solutions with compounds, that ordinarily do not appear in colloidal form. These methods admit of easy classification, in that they fall into two general classes—chemical methods and electrical methods.

The chemical methods include reduction methods, oxidation methods, hydrolytic methods, double decomposition methods, methods involving replacement of solvent, " protective methods," and " peptisation " methods.

Electrical methods have come prominently into play in recent years.

In all these methods all the apparatus must be absolutely clean.

208. Preparation by Chemical Reduction. By means of various reagents, such as, hydrogen gas, carbon dioxide, phosphorus, tannin, etc., the salts of the metals in very dilute solution are reduced in such a manner that the metals are held in suspension.

a. Preparation of colloidal silver solution. To 10 c.c. of a 1 per cent silver nitrate solution add ammonium hydroxide solution drop by drop, with constant stirring, until the precipitate which forms just redissolves. Dilute the solution with 200 c.c. of distilled water. Mix equal volumes of this solution with a 2 per cent tannic acid solution. A brown solution having a greenish color in reflected light will be formed.

NOTE.—Ascertain whether the same results are obtained by adding the tannic acid solution to the original silver nitrate solution.

b. Preparation of colloidal gold solution. Dilute 1 c.c. of commercial gold chloride solution with 50 c.c. of distilled water. In another beaker prepare a 0.5 per cent tannic acid solution. In one test-tube place 15 c.c. of the gold chloride solution and 5 c.c. of the tannic acid solution, then heat with constant shaking for a minute or two. A cherry red solution of colloidal gold is obtained. In another test-tube mix equal portions of the tannic acid and gold chloride solutions. Let stand in cold. A purple to a blue colloidal solution is obtained.

209. Preparation of Colloidal Solutions by Oxidation. Colloidal Sulphur. The formation of colloidal sulphur by oxidation of hydrogen sulphide seems to be the only instance of this method.

Roffo (Koll. Zeitsch., 1908, 2, 358) suggests the following method for preparing colloidal sulphur from sodium thiosulphate and sulphuric acid: Place 70 grams ($38\frac{1}{2}$ c.c.) of concentrated sulphuric acid in a 300 c.c. beaker. Set the beaker in cold water. Add *drop by drop* and with constant stirring a solution of sodium thiosulphate prepared by

dissolving 50 grams of the salt in 30 c.c. of distilled water. Hydrogen sulphide is first formed, then sulphur dioxide, followed by a whitish precipitate, which changes to lemon-yellow. When the action is over; add 30 c.c. of water; shake the mixture; and heat on a water-bath at 80° C. for 10 minutes. The sulphur dioxide is expelled, and the thick turbid mass clears up and assumes a sulphur-yellow color. Filter through glass wool to remove the undissolved sulphur. Set away in a cold place overnight. Reheat to 80° C. and refilter. Repeat this process as often as necessary. The solution is then centrifuged, the sediment washed in a little cold water, and then dissolved in as small a quantity of hot water as possible. On neutralizing with sodium carbonate the greater portion of the sulphur is precipitated, and a stable colloidal solution of sulphur remains. This contains a trace of sodium sulphate.

210. Preparation by Hydrolysis. The hydrolysis of salts is really a special case of double decomposition in which water is one of the substances involved. The reaction may be represented by the following equation:

$$\mathrm{Ct}'-\mathrm{An}^{\cdot}+\mathrm{H}'\mathrm{OH}^{\cdot} \rightleftarrows \mathrm{H}'-\mathrm{An}^{\cdot}+\mathrm{Ct}'-\mathrm{OH}^{\cdot},$$

where Ct' and $\mathrm{An}^{\cdot}$ stand for cation and anion respectively of the dissolved substance. When in the course of hydrolysis, one of the products is practically insoluble, conditions are favorable for obtaining a stable colloidal solution.

a. Preparation of colloidal ferric hydroxide. Place 100 c.c. of distilled water in a beaker and heat to boiling. To the boiling water add 1 c.c. of a filtered 33 per cent solution of ferric chloride. A reddish-brown solution is obtained, which contains colloidal ferric hydroxide and hydrochloric acid. It can be used for many experiments without purification. Most of the acid can be removed by dialysis. Write the equation.

211. Preparation by Double Decomposition. Under this heading we may include most of the chemical condensation methods, which are not reductions, oxidations, or

hydrolyses. One of the double decomposition products must be nearly insoluble.

a. Preparation of colloidal cadmium sulphide. Treat a very dilute solution of cadmium chloride with hydrogen sulphide. This solution gradually becomes milky and finally develops a yellowish color with reddish surface. The excess of hydrogen sulphide should be removed by boiling.

b. Preparation of colloidal arsenious sulphide. Boil about 1 gram of arsenious acid for a few minutes in 75 c.c. of distilled water. Filter the solution and allow to cool. On passing hydrogen sulphide through the cold solution, it gradually turns an orange-yellow color with a greenish surface.

c. Preparation of colloidal barium sulphate in aqueous alcohol. Dilute 10 c.c. of 2/1 normal sulphuric acid with 20 c.c. of alcohol (95 per cent), and add to it an equivalent amount of barium acetate, which has been diluted with 6 volumes of alcohol (2.56 grams of barium acetate dissolved in enough water to make the total 10 c.c. is then diluted with 60 c.c. of alcohol). A transparent gel of barium sulphate will be obtained. This gives a clear colloidal solution when diluted with a large quantity of water.

212. Preparation with a Protective Media. Many substances which, if formed in pure water, would tend to precipitate out, will be retained in a finely divided state of suspension provided some viscous media is present, viz., small quantities of gelatin, gum arabic, dextrin, starch, albumin, etc., seem to exert some sort of protective action of the small particles of lead chromate as they are formed, thereby preventing their clumping into larger masses. Again colloidal solutions of certain inorganic salts may be prepared by dissolving them in such fluids as glycerol and then pouring the solution into water.

a. Preparation of colloidal silver iodide with gelatin as the protective media. Mix in a small beaker 1 c.c. of a 0.5 per cent solution of gelatin and 10 c.c. of a 0.2 per cent solution of silver nitrate; in another beaker make a similar solu-

tion with gelatin and potassium iodide, then mix the two solutions slowly, and with constant stirring. A fine suspension of silver iodide will be obtained.

b. Preparation of colloidal lead chromate with glycerol as the protective media. Dissolve separately 0.5 gram portions of potassium chromate and lead nitrate in 10 c.c. portions of glycerol. Mix the solutions and pour into water. A colloidal solution of lead chromate is formed.

213. Preparation by Mechanical Dispersion. Certain substances, such as the natural gums, which are insoluble in water, may be converted into hydrosols (colloidal solutions in water) by dissolving them in some other liquid and then pouring into a large volume of water.

a. Preparation of a colloidal solution of gum mastic. Dissolve a small quantity of gum mastic in ethyl alcohol, then mix a few drops of the alcoholic solution with 100 c.c. of distilled water. A milky-white permanent colloidal solution of gum mastic is obtained. It is, however, very sensitive to electrolytes.

214. Preparation by "Peptisation." Under the influence of certain foreign substances (sometimes traces suffice) hydrogels or precipitates may be changed into hydrosols. The term "peptisation" was first suggested by Graham in allusion to the transformation of insoluble proteins into soluble compounds by pepsin.

a. Preparation of colloidal cadmium sulphide by peptisation. After making ammoniacal a solution of cadmium sulphate, add hydrogen sulphide until precipitation is complete. Thoroughly wash the precipitate by decantation with water. Finally, suspend the precipitate in distilled water and pass in hydrogen sulphide. The suspension becomes first milky and then a clear golden-yellow. Boil the solution until the hydrogen sulphide is driven off.

215. Preparation of Metal Sols by Electrical Dispersion Methods. (Bredig and Von Berneck's Method.) In this method the colloidal solutions are produced by causing an electric arc to pass below the surface of distilled water con-

tained in a very insoluble vessel. The method will be described in the case of metallic platinum.

a. Preparation of colloidal platinum. Dip two platinum wires of about 1 millimeter in diameter into distilled water, contained in a glass vessel of good quality. Bring the wires close together, *but do not let them touch.* Pass a current of from 8–10 amperes and 30–40 volts, through the wires. The metal will be torn off from the cathode in a very finely divided state, and the water will soon become dark brown in color. After the solution has assumed a deep color, it should be filtered through a folded hardened filter paper to remove any large particles of platinum which may have been torn off.

C. Identification of Colloids

216. True Solutions, Mechanical Suspensions, and Colloidal Solutions. A chemically heterogeneous liquid, i.e., one which can be separated by changes in temperature and pressure (distillation, freezing, etc.) into at least two components of different chemical composition, may appear (as pointed out by Ostwald in his Handbook of Colloids) in any one of the three following states:

" *a.* The unknown solution may be an ordinary or 'true' (molecular-disperse) solution of one or more substances. *b.* It may be a coarse 'mechanical suspension' of one or more substances which form true solutions to a limited degree only, if at all. *c.* It may be a colloidal solution."

217. Properties of Mechanical Suspensions. A typical mechanical suspension may be made by thoroughly shaking about 1 gram of finely ground quartz or kaolin in 100 c.c. of water and pouring off the large particles which quickly settle.

The following observations may be made on a kaolin suspension prepared as indicated above, or on a solution suspected of being a mechanical suspension.

a. Is it turbid?

b. Fill a test-tube with the mixture and set it aside undisturbed for half an hour, and then observe if sedimentation has taken place. Repeat your observation at the end of 24 hours. Record results.

c. Make a microscopical examination with different magnifications. Note the number of visible particles in the field in each case.

d. Centrifuge a portion of the mixture for 5 minutes and then repeat the microscopical observations with samples from the top and bottom of the centrifuge tube.

e. Filter the suspension, using different grades of filters.

A good grade of filter paper holds back particles having a diameter greater than about 5 microns (a micron is a thousandth of a millimeter); a hardened filter paper holds back particles about 2 microns in diameter. Certain clay filters will hold back particles of from 0.2 to 0.4 microns in diameter.

218. Distinguishing Characteristics of a Colloidal Solution. When the suspended particles are in a very finely divided state, the results obtained by the experiments outlined in Article 217 will be indefinite or negative. Under such circumstances the liquid under examination must be either a *true solution* or a colloidal solution.

Unless otherwise directed, use a colloidal ferric hydroxide solution in making the following observations:

a. Does the solution appear to be homogeneous?

b. Do you notice any appreciable change in homogeneity when some of the solution is diluted with 2, 4, or 8 volumes of distilled water?

c. Turbidity. Place some of the colloidal solution in a perfectly clean, thin-walled test-tube, and hold it against an opaquely black background, e.g., black paper, or better, black velvet. If the solution is colorless, but turbid the background shining through it assumes a grayish-white appearance. If the solution is colored, it should be highly diluted.

d. Tyndall phenomenon. Place the liquid in a thin-

walled beaker, or better, a thin-walled rectangular glass cell; make the room as dark as possible, then project a strong, slightly convergent beam of light through the liquid. (The light of a small arc concentrated by a lens will be found satisfactory). On looking at the vessel from the side it will appear turbid in the path of light. If a converging lens is used, the turbidity will be cone-shaped. Make a " control " test with distilled water, that has been specially prepared and protected from dust as much as possible (" optically empty " water is very difficult to prepare).

While the beam of light is passing through the control water, introduce a little of the colloidal solution under examination into it, and note the increased turbidity.

e. Visibility of the particles. The ultramicroscope and related instruments. Since the particles in a colloidal solution are of submicroscopic dimensions, it is only with favorably arranged illumination that they can be rendered visible. The so-called ultramicroscope of Siedentopf and Zigmondy gets over this difficulty. The principle of this instrument is very simple; a strong beam of light is projected horizontally through a very thin layer of the colloidal solution, which is observed through a microscope. The particles reflect the light into the microscope and appear as colored discs.

Instead of the ultramicroscope, which is rather expensive several simpler instruments have been invented, which will show the particles of many colloidal solutions by reflected light. The principle of all these appliances is similar. Paraboloid or cardiod condensers are used with an ordinary microscope, the particles being rendered visible by means of a small arc or a Nernst lamp. The beam of light is made to pass by reflection into the condenser, which contains a small quantity of the sol.

By means of one of the instruments described above examine several colloidal solutions.

f. Brownian movement of particles. Note that the particles visible in the ultramicroscope, or allied instruments show ceaseless Brownian movement.

g. Diffusion through dilute gels. Half fill 8 test-tubes with hot 3 per cent gelatin solution and allow it to congeal. Label and number the test-tubes, and then nearly fill them with the solutions named below:

1. A 1 per cent aqueous solution of safronin.
2. A 1 per cent aqueous solution of Congo red.
3. A 1 per cent aqueous solution of picric acid.
4. A 2 per cent aqueous solution of copper sulphate.
5. A 2 per cent solution of ferric chloride.
6. Colloidal silver solution (Exp. 208, *a*).
7. Colloidal ferric hydroxide (Exp. 210, *a*).
8. Colloidal cadmium sulphide (Exp. 214, *a*).

Observe and make notes on the degree of diffusion in each of these tubes at the end of 1 hour, 1 day, and 1 week.

h. Dialysis. Prepare 10 dialyzers by one of the following methods:

Method I. Carefully tie a piece of moist parchment paper over the end of a piece of glass tubing, about 15 mm. in diameter and 100 mm. in length. Test the tubes for leakage. (The smallest pinhole would vitiate the results.) Tie a piece of string around each tube so as to make a loop at the top; pass a stirring-rod through the loop and then suspend the tubes in wide-mouthed bottles or very large test-tubes.

Method II. Collodion sacs. Into a clean, dry, medium sized test-tube pour about 10 c.c. of collodion, then pour it back into the stock bottle, revolving the tube with the mouth downwards so that an even film is left adherent to the walls of the tube. Add another portion of collodion and repeat as before. Allow the film to dry, so that it does not stick to the finger. At this stage fill the tube with cold water. Cut the rim of the tube with a knife, and carefully detach a little of the film from the tube. Run in a little water between the film and the glass. By means of a small glass rod the sac can be partly pushed away from the glass. Add more water between the sac and the glass, and again

use the glass rod to pry away the sac. Finally pull the sac out of the tube. After a little practice one can *usually* remove the sacs from the tubes in perfect shape. Fill the sac with water. It should be perfectly transparent. Keep the sacs in water until ready for use, as on drying they become porous. Tie a cork with a large hole in it into the upper end of the sac. By making a loop with the string the sac can be suspended in wide-mouth bottles, etc. These sacs are better than the parchment tubes.

NOTE.—Preparation of Collodion Solution. To 3 gms. of gun cotton (pyroxylin) in an Erlenmeyer flask add 75 c.c. of ether. Cork, and let stand 15 minutes. Then add 25 c.c. of ethyl alcohol, gently rotate, and let stand until all the bubbles have disappeared. Keep well corked in a cool place.

Method III. Collodionized paper capsules. Soak filter-paper capsules in collodion solution; drain off the collodion and carefully hold the tube until the collodion hardens. The tube should then be kept in distilled water until you wish to use it. A cork with a large hole can be tied into this thimble in the same manner as indicated in Method II. This type of dialysis tube is very satisfactory, but relatively expensive.

Into the tubes, prepared by one of the methods described above, place 15 c.c. of the solutions named below:

a. White of egg, 1 part of dried egg white in 20 parts of water (filtered).

b. Witte's peptone, 1 per cent aqueous solution.

c. Dextrose, 1 per cent aqueous solution.

d. Dextrin, 1 per cent aqueous solution.

e. Soap, 1 per cent aqueous solution of neutral soap.

f. Butyric acid, 0.1 per cent aqueous solution.

g. Sodium chloride, 0.1 per cent aqueous solution.

h. Gelatin, 1 per cent aqueous solution.

i. Ferric hydroxide sol., prepared as outlined in Exp. 210, *a.*

j. Silver sol (see article 208, *a*).

Suspend the tubes in wide-mouth bottles or very large test-tubes and surround each tube with enough distilled water to bring the outside and inside liquids to the same level. Occasionally raise and lower the tubes gently to favor diffusion.

Diffusion Results. The diffusion should be continued for about an hour. At the end of this time test the diffusates as follows:

a. Test the diffusate from (*a*) for albumin by applying the coagulation test (Exp. 191), and the biuret test (Exp. 193, *a*).

b. Apply the biuret test to some of the diffusate from (*b*); also to some of the solution from the inside of the tube.

c. Test the diffusate from (*c*); also the solution from the inside of the dialyzer for dextrose with Fehling's solution. (Exp. 99, *a*).

d. Test the diffusate from (*d*) as follows:

1. Apply the iodine and potassium iodide test (Exp. 114).
2. Test with Fehling's solution to ascertain whether any admixed reducing sugar has dialyzed.

Apply these same tests to the solution in the dialyzer and compare the intensity of the reaction with the results obtained from the diffusate.

e. Answer the following questions regarding both the diffusate and the solution from the inside of the dialyzer in Exp. (*e*):

1. Is it frothy?
2. Does it yield a white flocculent precipitate with calcium chloride solution?
3. Does it form an emulsion when shaken with a drop of cottonseed oil?

f. Is diffusate (*f*) acid to litmus?

g. Does diffusate (*g*) give the chloride reaction with silver nitrate solution?

h. Does diffusate *h* give off the smell of ammonia when a small portion of it is heated with a few drops of sodium

hydroxide solution? Does the diffusate give a positive biuret reaction?

i. Is the diffusate *i* reddish-brown in color?

j. Is the diffusate *j* brown in color?

From the data obtained from your experiments which of the above solutions would you designate as colloidal?

i. Preparation and application of an "Untrafiltration" apparatus. Select a filter paper capsule that *fits* a No. 5 rubber stopper. Collodionize the capsule according to the directions outlined in Exp. 218, *h,* Method III. After the collodionized capsule has been prepared and left in distilled water, select a suction flask that fits a No. 7 rubber stopper and arrange the apparatus as follows: Place a No. 7, one-hole, rubber stopper on the stem of a funnel; further down on the stem place a No. 5, one-hole rubber stopper. Tightly tie the collodionized capsule to the No. 5 stopper. Place the funnel in the suction flask, which, in turn is attached to a suction pump. Finally place the colloidal solution in the funnel and apply suction.

Perform this experiment with a colloidal solution of silver, or ferric hydroxide. Do you obtain a colored filtrate? How does the turbidity of the filtrate compare with the liquid that remains inside the collodion sac and with the original solution?

219. Classification of Colloids. From the experiments outlined above, it is evident that colloids differ from coarse suspensions in that the particles are not visible with an ordinary microscope, and in that they can pass through even the best grade of filter papers. The experiments also convince one that colloids differ from "true" or molecular solutions in that they do not readily diffuse, and do not dialyze through vegetable or animal membranes, whereas most molecular solutions do. In other words, the degree of subdivisions is greatest in molecular solutions and least in the coarse suspensions. Colloidal solutions occupy a middle position.

The fluid in which the "particles" are suspended is

called the *dispersion medium*, or *external phase*, and the particles themselves are called the *dispersoids*, or *internal phase*.

It has been found necessary to " fix " the limits of the size of the particles that will be accepted as belonging to the colloidal state. Since the limit of the microscope vision is about one ten-thousandth of a millimeter, or 0.1_u, this value is arbitrarily taken as the dividing line between the coarse suspensions and colloidal dispersoids. Now, true molecular dispersoids (see texts on Physical Chemistry) have diameters of one ten-millionth to one one-millionth of a millimeter (0.1 to $1_{\mu\mu}$). Hence, one one-millionth of a millimeter is accepted as the dividing line between colloidal particles and molecular dispersoids. See the following diagram:

Dispersed Systems. (From Ostwald.)

↓ Coarse Dispersoids	↓ Colloids	↓ Molecular Dispersoids
Particles greater than 0.1^{μ}; do not pass through filter papers; microscopically analyzable.	Increase in degree of dispersion 0.1_{μ} to $1_{\mu\mu}$ Pass through filter papers; cannot be analyzed microscopically; do not diffuse or dialyze.	Particles smaller than $1_{\mu\mu}$; pass through filter paper; cannot be analyzed microscopically; do diffuse and dialyze.

The three divisions are further divided as follows:

1. Coarse dispersoids; suspensions and emulsions.
2. Colloids; suspensoids and emulsoids.
3. Molecular dispersoids; crystalloidal solutions, both electrolytes and non-electrolytes.

Suspensions and suspensoids are systems in which solid, non-deformable particles are suspended in a liquid.

Emulsions and emulsoids are systems of two liquids insoluble in each other; they consist of liquid deformable particles of one liquid suspended in another liquid with which they do not mix.

Most of the natural colloidal solutions are emulsoids, and most of the artificial colloidal solutions are suspensoids.

D. Viscosity of Colloidal Solutions

220. Estimation of the Viscosity of Suspensoids and Emulsoids. After thoroughly cleansing a Mohr's pipette, clamp it in an upright position and attach to the top a piece of rubber tubing, which contains a piece of capillary tubing to control the outflow. Make the following tests:

a. Fill the pipette to the zero mark with distilled water and carefully record the time required for the liquid to flow from the zero mark to the 10 c.c. mark. Repeat this four or five times and average the results.

b. Rinse the pipette with a kaolin suspension and again record the time required for the liquid to flow from zero to the 10 c.c. mark. Repeat four times and average the result.

c. Follow the same technique with the following colloidal solutions:

Suspensoids—arsenic sulphide and ferric hydroxide.
Emulsoids—starch paste, 1 and 5 per cent; egg white, 1 per cent; gelatin, 1, 5 and 10 per cent; soap solution, 5 per cent.

Calculate the viscosity of each solution as compared with water by means of the following formula:

$$V_s = \frac{(t \times d)_s}{(t \times d)_{H_2O}}$$

where V_s is the relative viscosity of the solution which we wish to obtain, t is the time of outflow, and d is the density.

What do you conclude in regard to the viscosity of suspensoids and emulsoids?

(*d*) Cool some distilled water, a suspensoid solution, and an emulsoid solution by placing beakers containing these liquids in a vessel containing cracked ice. Estimate the density and the temperature of each and determine the

relative viscosity of each solution as quickly as possible. Compare your results with water at room temperature.

What is the relative effect of decrease of temperature on the viscosity of pure solvents, suspensoid sols, and emulsoid sols?

E. Electrical Properties of Colloids

221. Electrical Migration of Suspensoids (Cataphoresis). Prepare a pair of platinum electrodes as follows: Insert insulated copper wire through a narrow piece of glass tubing about 4 inches long. Fasten a short thin piece of platinum wire to the copper wire and draw the joined part into the glass tube. Seal the platinum wire into the tube, and then make a flat helix with most of the platinum wire that remains outside the tube.

Introduce the electrodes into the arms of a U-tube which is three-quarters filled with colloidal ferric hydroxide solution, and attach the terminals of the electrodes with a series of battery cells, or better, with the electric lighting system. Note what happens as soon as a current is passed. Continue your observations for some time, and make notes on what you observe.

Repeat with colloidal arsenious sulphide.

Did both the colloids migrate to the same pole?

Introduce a commutator into the system; change the direction of the current; and with fresh portions of the same colloidal solutions repeat the above experiment. Explain the results you obtain.

222. Electrical Migration of Emulsoids. Repeat Exp. 221, using amphoteric, acid, and alklaine egg albumin solutions (1 per cent). Record your results.

223. Electrical Endosmosis. This term is applied to the passage of a liquid through a diaphragm, or through a capillary tube, towards one of the electrodes, when a current of electricity is passing.

The following methods will be found very satisfactory for quick qualitative experiments:

Method I. By means of a colloidal diaphragm. Make a $2\frac{1}{2}$ per cent solution of agar, and filter it hot through a moistened wad of cotton or muslin. Add a small quantity of Congo-red solution to the filtered agar, and then pour a little of it (enough to cover the bend) into several U-tubes and allow it to set. Note: Agar makes a better diaphragm than gelatin, as it is not so sensitive to heat. Fill both the arms of one of these U-tubes to the same level with the colloidal solution to be tested. An inch and a half from the top is a good height. Introduce platinum electrodes, similar to those described in Exp. 221, into the arms of the U-tubes and note what happens on passing a current for some time. The experiment requires from 15 minutes to 1 hour (the time varies with the colloid used) when a strong current such as the electric lighting system is used. Avoid overheating, as the agar will melt and vitiate the result. By observing the change in color in the indicator in the agar the relative concentration of H-ions and the direction in which they travel can be ascertained.

a. Perform the experiment with ferric hydroxide sol- and arsenious sulphide sol. Does the liquid rise in the same arm of the U-tube in both cases? If so, which pole is it? By means of a commutator change the direction of the current and repeat the experiment with fresh portions of sols. Now what happens?

b. Repeat with a 1 per cent amphoteric egg-white solution. Do emulsoids act the same as suspensoids?

Method II. By means of capillaries. Select a piece of bending tubing of about $\frac{1}{2}$ inch inside diameter. Cut off a piece of about 12 inches in length, and bend into a U-tube. The arms should be about $4\frac{1}{2}$ inches in length. Cut the U-tube in two, and then connect the short arms by means of two one-hole rubber stoppers, through which a capillary tube about 3 inches long has been inserted. See diagram. Three-quarter fill both arms of the U-tube with the colloidal solution to be tested. Introduce platinum electrodes, similar to those described in Exp. 221, and note what hap-

pens when a current is passed for some time. Mark the top level of the liquid in the tubes with a wax pencil in order to note any rising or lowering of the liquid. Observe the capillary tubes by placing a light on the opposite side. Record your observations.

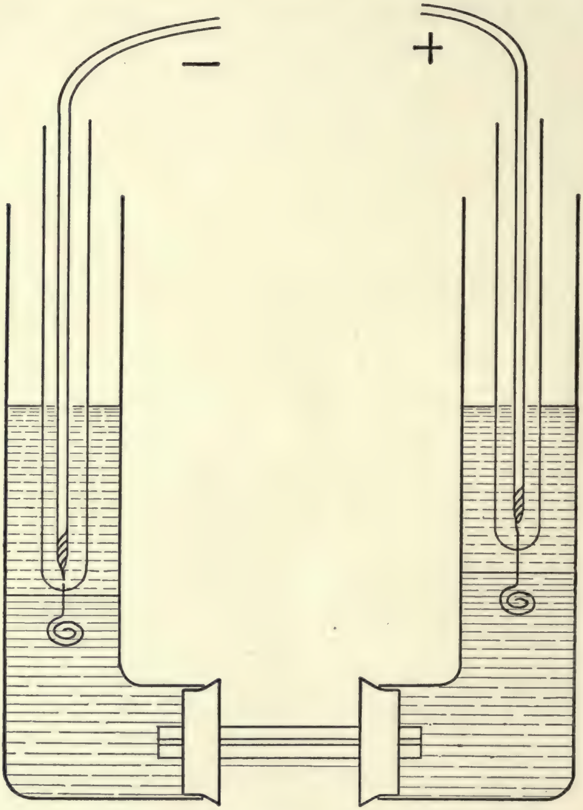


Fig. 2.—Capillary Apparatus for Electrical Endosmosis.

F. Precipitation of Colloids by Electrolytes

224. Action of Electrolytes on Suspensoids. Prepare a colloidal solution of gum mastic (see Exp. 213, *a*), and to 10 c.c. portions of the filtered gum mastic sol. add the following substances:

a. 5 c.c. of M/10 HCl.

(M is used to represent molar concentration, i.e., molecular weight of a substance expressed in grams dissolved in enough water to make the total volume 1000 c.c.).

b. 5 c.c. of M/10 NaOH.

c. 5 c.c. of M/1 NaCl.

d. 5 c.c. of M/20 urea.

e. 5 c.c. of M/20 glucose.

Let stand for half an hour. Note what occurs and explain.

225. Effect of the Concentration of the Electrolytes on the Precipitation of Suspensoids. By means of a Mohr's pipette, graduated in tenths, prepare a series of 7 test-tubes as follows:

To tube 1 add 10 c.c. of M/10 NaCl.

To tube 2 add 5 c.c. of M/10 NaCl and 5 c.c. H_2O (equal M/20).

To tube 3 add 2 c.c. of M/10 NaCl and 8 c.c. H_2O (equal M/50).

To tube 4 add 1 c.c. of M/10 NaCl and 9 c.c. H_2O (equal M/100).

To tube 5 add .5 c.c. of M/10 NaCl and 9.5 c.c. H_2O (equal M/200).

To tube 6 add .2 c.c. of M/10 NaCl and 9.8 c.c. H_2O (equal M/500).

To tube 7 add 10 c.c. of distilled water (control).

Introduce 0.5 c.c. of *dialyzed* ferric hydroxide sol. into each tube. Do you observe any immediate changes in the aspect of the tubes? After 10 minutes do you observe any changes in appearance?

Preserve these test-tubes for future experiments.

226. The Precipitating Effect of Various Anions on Suspensoids. Prepare similar series of 7 tubes (i.e., the same concentrations used in Exp. 225) with each of the following salt solutions: sodium bromide; sodium iodide; sodium sulphate; and sodium phosphate (Na_2HPO_4). Add to each tube 0.5 c.c. of dialyzed ferric hydroxide sol.

Note what occurs, compare with the results obtained in Exp. 225, and then write up your conclusions in regard to the effect of anions on suspensoids.

227. Precipitating Effect of Mono-, Di- and Trivalent Cations. Take three separate portions of 10 c.c. each of a colloidal solution (e.g., ferric hydroxide sol.) and to each portion add one of the following solutions from a burette: M/10 potassium chloride, M/10 barium chloride, and M/10 aluminum chloride. Determine the amount of these electrolytes which will just completely precipitate the colloidal particles. This should be done roughly at first in order to get an idea of the approximate amount required, and then it should be repeated more carefully. Allow the precipitate to settle after each addition, and note the effect of the next addition in the supernatant liquid, which should gradually become colorless. Do you notice any difference in the precipitating power of cations of different valence? Explain.

228. Adsorption of the Precipitating Ion. Thoroughly wash the precipitates obtained in Exp. 227 with distilled water and then test the residue for the cations. Can the *cation* be removed from the colloidal precipitate by washing? Is the *anion* present in the washed precipitate? Explain.

229. Effect of Heat on the Concentration of Ions Required to Effect Precipitation of Suspensoids. Place the tubes of Exp. 225, which showed little or no sign of precipitation in a water-bath, and carefully heat nearly to boiling. Does this effect the precipitation of any of them?

230. Behavior of Electrolytes on Emulsoid Sols. *a.* Place 2 c.c. of a 1 per cent neutral soap solution into each of three test-tubes. Add to these respectively saturated solutions of sodium chloride, magnesium sulphate, and ammonium sulphate until precipitation takes place. Add the salt solutions from graduated cylinders and note the quantity necessary to effect maximum precipitation in each case.

b. Using the same salt solutions repeat the above experi-

ment with a 1 per cent starch paste and a 1 per cent gelatin solution. Record your results.

Does the valency of the precipitating ion manifest the same phenomenon with the emulsoids that it does with suspensoids?

G. Mutual Precipitation and Protection of Colloids

231. Mutual Precipitation of Suspensoids. Mix equal volumes of colloidal solutions of ferric hydroxide and arsenious sulphide (use 5 c.c. of each). Make a note of what occurs.

232. Effect of Varying the Relative Concentration of the Sols. Having Opposite Signs. Prepare a colloidal solution of antimony sulphide (see Exp. 211) of such a strength that each c.c. will contain about 3 mgs. of the colloid. Place 2 c.c. of this solution into each of 8 test-tubes. Add to these tubes respectively 7.5, 7, 6, 5, 4, 3, 2, and 1 cc. of distilled water and 0.5, 1, 2, 3, 4, 5, 6, and 7 c.c. of dialyzed colloidal ferric hydroxide solution. Mix thoroughly, and then record your results in tabulated form, using the following terms to describe the result: no change, cloudy, small flakes, large flakes, complete precipitation, slight precipitation, etc. Make your observations directly after mixing and again after the lapse of an hour.

233. Mutual Precipitation of Suspensoids and Emulsoids Having Opposite Charges. To 5 c.c. of egg-albumin (1 per cent) solution add an equal volume of ferric hydroxide sol. Note the appearance of the mixture and then add about 0.5 gram of sodium sulphate and shake the mixture thoroughly. A brownish mass containing the protein and an excess of the ferric hydroxide sol. is precipitated. Does the filtered solution contain protein? Perform the biuret and Millon's tests to ascertain this data.

234. Protective Action of Emulsoids on Suspensoids. Introduce into a test-tube 1 c.c. of gum mastic sol. (see Exp. 213), 1 c.c. of 0.1 N $MgSO_4$, and 1 cc.. of distilled water in the order named. In another test-tube introduce

1 c.c. of gum mastic sol., 1 c.c. of 0.1 N $MgSO_4$, 2 drops of a 1 per cent gelatin solution, and 1 c.c of water in the order named. Observe the results directly after mixing; after 15 minutes; and after 24 hours. Explain.

H. The Phenomenon of Adsorption by Colloids

235. General Remarks. It has long been known that charcoal has the power to take up certain gases like carbon dioxide in large quantities. It can also adsorb certain constituents from solutions. This fact has been utilized to advantage in such processes as the purification of sugar. The coloring matter from the solutions of crude sugar is removed by charcoal, and a colorless product results.

236. Adsorption of Solutes by a Solid Adsorbent. *a.* Determine the concentration of solutions of acetic acid, succinic acid, and hydrochloric acid (furnished by the instructor) with N/20 sodium hydroxide solution. Express the concentrations in *moles per liter.* Use phenolphthalein as an indicator. Use the same amount of the indicator in each test, say, 2 drops for each 10 c.c. of acid taken.

To 50 c.c. portions of each acid add 2 grams of powdered animal charcoal and stir the mixtures thus obtained for about 20 minutes. Then filter on a hardened filter paper. Do not wash the precipitate. Finally, determine the concentration of the filtrate in moles per liter by titrating several 10 c.c. portions with N/20 NaOH. Tabulate your results under the following headings:

	C_1	C_2				
		1	2	3	4	Average.
Acetic acid	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .
Succinic acid	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .
HCl	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .

In the preceeding table C_1 represents the original concentration in moles per liter and C_2 the molar concentration after adsorption has taken place.

What do you conclude in regard to the adsorption of slightly ionized acids as compared with highly ionized acids?

b. Adsorption of alkalies. To two portions of N/20 NaOH of 25 c.c. each add 2 grams of animal charcoal and treat as above. Use the hydrochloric acid used in the first experiment to ascertain the quantity of sodium hydroxide adsorbed, since you know its concentration from your former titration. Record your results as before.

What do you conclude from this experiment in regard to the adsorption of highly ionized alkalies as compared with highly ionized acids?

c. To 10 c.c. of an aqueous solution of fuchsine add 1 gram of powdered charcoal, and stir repeatedly for 20 minutes. Filter on a hardened filter paper. Is the filtrate colored? Add some alcohol to the charcoal on the filter paper. Is *this* filtrate colored? Explain.

d. Repeat Exp. *c* with another adsorbent, for example, powdered kaolin. Does charcoal alone possess the property of adsorbing substances in solution?

237. Effect of Temperature on Adsorption. Repeat Exp. 236, *a*, which was performed at room temperature, i.e., at about 25° C., at a temperature of 75° C. What do you conclude from this experiment in regard to the effect of temperature on adsorption?

I. Special Properties of Emulsoids

238. General Remarks. The word " Emulsoid " indicates the liquid nature of the dispersed phase, but, since this phase may contain a greater or less percentage of water, or other solvent, with the same composition of the actual solid matter itself, as is especially the case with proteins, it is evident that all degrees may exist between solid and liquid.

Silicic acid is one of the few examples of an inorganic emulsoid. There are many examples of organic emulsoids, and it is this class of colloids that are of most importance to biologists. The organic emulsoids include gelatin and agar, proteins, gum-arabic, cellulose and nitro-cellulose solutions.

239. Preparation and Properties of Silicic Acid Gel and Sol. Either prepare, or obtain from the instructor, 50 c.c. of a 10 per cent solution of sodium silicate. On mixing some of this water glass with some 2N HCl a stiff gel is formed. In order to determine the correct relations for mixing a trial experiment must be performed. Run the water glass into a definite amount of the hydrochloric acid, say 10 c.c. until the solution begins to solidify. Shake continually while the addition is being made. To prepare the hydrosol add about two-thirds of the water glass necessary to produce the hydrogel. This can be purified by dialysis.

Ascertain if the solid hydrogel is soluble in water, dilute acids, and alkalies. Does the rigid gel lose water on exposure to the air? Is there any change in the physical appearance of the gel on drying?

240. Swelling and " Solution " of Typical Organic Emulsoids. Examine samples of dry gelatin, agar, egg-albumin, blood-fibrin, casein, gum-arabic, dextrin, and starch. Place about 0.5 gram of each of these substances in separate test-tubes, and then add 10 c.c. of water. Allow the tubes to stand at room temperature for an hour. Note carefully the appearance of each substance. Place them all in a beaker of water and gradually heat until the water comes to the boiling-point, and again note and record the appearance of each.

241. Preparation of Pure Salt-free Albumin by Hofmeister's Method. " Carefully separate the egg-white from the yolks of two or more eggs (perfectly fresh eggs give the best results), then mix equal volumes of egg-white and saturated ammonium sulphate. Beat to a stiff froth, and let stand overnight. Filter off the precipitate of globulin and

mucoid, and add gradually to the clear filtrate, with gentle stirring, a 10 per cent solution of acetic acid, until a white precipitate is formed. This mixture should be just acid to litmus. Then add 1 c.c. of the 10 per cent acid for each hundred c.c. of solution; a bulky precipitate is formed, which becomes crystalline in 5 hours. Allow to stand until the next day to obtain the full yield (60 grams per liter). To purify: Filter, and wash the precipitate three times with half saturated ammonium sulphate solution containing 0.1 per cent of acetic acid. Dissolve the crystals in the minimum of water, and add half saturated ammonium sulphate solution until a precipitate is formed; then add 2 c.c. more to each liter. The albumin recrystallizes in 24 hours. Repeat this treatment as often as necessary. Finally, the crystals are dissolved in pure water, and dialyzed to remove the ammonium salts."

242. Coagulation of Pure Salt-free Albumin by Heat. Introduce 1 c.c. of a 1 per cent solution of salt-free albumin into each of 5 test-tubes. To the first add 5 c.c. of distilled water; to the second add 5 c.c. of distilled water and 2 drops of N/10 HCl; to the third add 4 c.c. of distilled water and 1 c.c. of N/10 HCl; to the fourth add 5 c.c. of distilled water and 2 drops of N/10 NaOH; and to the fifth add 4 c.c. of distilled water and 1 c.c. of N/10 NaOH. Tie the tubes in a bundle and suspend in a large beaker of water. Heat the water to boiling. Note whether coagulation takes place in all of the tubes, and in those which do coagulate note the temperature at which the coagulation takes place. (The thermometer should be placed in a test-tube containing an equal volume of water and tied in the bundle with the rest of the tubes.) Ascertain in which tubes coagulation is *complete* by filtering on a hardened filter paper and testing the filtrates for albumin, i.e., to portions of the filtrate apply biuret and Millon's color tests. Is the coagulated albumin soluble in water?

243. The Effect of Salts on Heat Coagulation. Introduce into each of 3 test-tubes 5 c.c. of the same salt-free

albumin solution used in Exp. 242, since you know its coagulation temperature. Mark these tubes *a*, *b*, and *c*, for convenience. Into tube *a* add 5 c.c. of 0.02 N sodium chloride solution; into tube *b* introduce 5 c.c. of 0.04 N sodium chloride solution; into tube *c* introduce 5 c.c. of 0.08 N sodium chloride solution. It must be borne in mind that the sodium chloride solution is reduced in concentration by mixing it with albumin solution. So, after mixing, the normality of the salt solution is just half the normality of the original salt solution, i.e., after mixing tube *a* is 0.04 N as regards the salt, tube *b* 0.08 N, and tube *c* 0.016 N.

Now determine the coagulation temperature of these tubes, and record your results in the table below.

Repeat the above experiment, using in tube *a* 0.02 N, in tube *b* 0.04 N, and in tube *c* 0.08 N *sodium acetate solution.*

TABLE

SALT.	FINAL CONCENTRATION OF THE SALT.		
	0.04 N	0.08 N	0.016 N
NaCl	....	....	....
CH_3COONa......	....	....	....

244. Coagulation of Albumin by Alcohol. Introduce into each of 2 test-tubes 5 c.c. of a 1 per cent salt-free albumin solution. To one of these tubes add 15 c.c. of alcohol and to the other add 1 c.c. of N/10 NaCl and a similar amount of alcohol. Does coagulation take place in both cases? Do electrolytes seem to be essential? Is the coagulated albumin soluble in water? Allow some of the coagulated albumin to remain under alcohol for 24 hours and then again test its solubility in water. Explain the result you obtain.

245. Syneresis. It has been shown by Graham, Ostwald and others, that, when any gel is left to itself for a

number of hours or days, protected against infection with microorganisms and against evaporation, *a separation into two phases takes place.* Graham called this separation *syneresis.*

Is the liquid given off from aqueous colloidal sols just water? Or is it a second, more dilute, colloidal solution which is secreted by the concentrated colloid?

Sterilize four small wide-mouth bottles and the corks to fit them. Label these bottles *a*, *b*, *c*, and *d*. Half fill them with the following gels: Bottle *a*, 5 per cent gelatin; bottle *b*, 2 per cent agar; bottle *c*, 5 per cent starch; bottle *d*, silicic acid gel (see Exp. 239). As soon as the gels are cooled to about room temperature, stopper the bottles carefully, and then set them aside in a cool place for 24 hours. If syneresis has taken place by this time, examine the " serums " as follows:

Pour off some of the " serum " from bottle *a* into a test-tube, add a few drops of HCl and some tannic acid solution. The development of a white cloud indicates the presence of gelatin. A biuret test on some of the fluid will confirm the conclusion.

Apply the HCl-tannic acid test to some of the " serum " from bottle *b*. Do you get a white cloud?

Apply the iodine-potassium iodide test (Exp. 114) to some of the " serum " from bottle *c*. Record result.

Add some copper sulphate solution to some of the " serum " from bottle *d*. The appearance of copper silicate shows that this fluid contained a colloid also.

246. The Effect of Electrolytes upon the Swelling of Dried Gelatin Plates. Prepare gelatin discs as follows: Fill a Petri dish with a 20 per cent solution of gelatin and allow it to set. As soon as it is completely set cut into discs of uniform size by means of a cork-borer (if the gelatin was perfectly uniform in thickness, it will not be necessary to weigh these discs for qualitative work). Allow the discs to dry at room temperature before using. To render the discs readily visible, a trace of a colloidal dye, such as Congo red,

may be added to the gelatin while it is hot. Place one of these discs in an equal quantity of each of the following solutions: Water; N/20 HCl; N/20 NaOH; N/2 KI; N/5 $CaCl_2$; and saturated $MgSO_4$ solution. Set aside for 24 hours and then observe and record your findings, i.e., indicate the order in which the substances have favored swelling.

247 Influence of Acid upon the Swelling of Gels. Select 6 test-tubes of uniform diameter, and place in each of them either 0.5 gram of granular gelatin, or 0.2 gram of finely ground blood-fibrin. Label these tubes *a*, *b*, *c*, *d*, *e*, and *f*. Place in five of them respectively 20 c.c. of N/10, N/50, N/100, N/200, and N/250 HCl. In the sixth tube place the same amount of distilled water. Shake these tubes by gentle rotation at frequent intervals during the remainder of the laboratory period. At the expiration of 2 *hours* and 24 *hours* make note of the changes that take place in the appearance of the gelatin or fibrin in the tubes. What concentration of acid affects the swelling to the greatest extent?

248. Influence of Alkalies upon the Swelling of Gels. Repeat Exp. 251, using the same concentrations of NaOH instead of the HCl. Do alkalies likewise affect the swelling of gels? How does the concentration which affects the swelling to the greatest extent compare with the concentration of acid which has the greatest effect?

249. Influence of Anions and Cations upon Swelling Induced by Acids. Select 12 test-tubes of uniform diameter and add to each of them either 0.5 gram of granular gelatin or 0.2 gram of finely powdered blood-fibrin. To eleven of these tubes add 20 c.c. of N/100 HCl. To ten of the tubes containing acid add respectively 5 c.c. of molar solutions of sodium bromide, sodium chloride, sodium tartrate, sodium sulphate, sodium phosphate, calcium chloride, barium chloride, magnesium chloride, ferric chloride, and aluminum chloride. To the eleventh tube add 5 cc. of distilled water. In the twelfth tube place 25 c.c. of distilled water. Gently

rotate all the tubes occasionally for the remainder of the laboratory session. Keep them for 24 to 48 hours. Record and compare your results.

250. Influence of Anions and Cations upon Swelling Induced by Alkalies. Repeat Exp. 249, using N/100 NaOH instead of the N/100 HCl. Record and compare your results.

Judging from your results, which have the most effect on swelling-anions or cations?

251. Further Data upon the Swelling of Gels. The increase in volume when materials swell can be demonstrated very clearly in the following fashion: Select 5 rubber bands of uniform size. Place one in a test-tube containing 10 c.c. of benzene, place another in a test-tube containing 10 c.c. of ether, place another in a test-tube containing 10 c.c. of petroleum ether, place another in a test-tube containing 10 c.c. of alcohol, and another in a test-tube containing 10 c.c. of water. Allow these tubes to stand about half an hour and then examine them. Note that benzene and ether are very effective in producing a gel out of rubber through swelling. Water has no effect.

Introduce a gelatin disc into benzene and another into ether. Let them stand for a while. Do you get any swelling?

After performing this experiment, do you think it would be desirable to use a rubber stopper in an ether bottle or a benzene bottle?

APPENDIXES

APPENDIX A—TABLES

TABLE I. INTERNATIONAL ATOMIC WEIGHTS, 1916

	Symbol	Atomic weight		Symbol	Atomic weight
Aluminum	Al	27.1	Molybdenum	Mo	96.0
Antimony	Sb	120.2	Neodymium	Nd	144.3
Argon	A	39.88	Neon	Ne	20.2
Arsenic	As	74.96	Nickel	Ni	58.68
Barium	Ba	137.37	Nitrogen	N	14.01
Bismuth	Bi	208.0	Osmium	Os	190.9
Boron	B	11.0	Oxygen	O	16.00
Bromine	Br	79.72	Palladium	Pd	106.7
Cadmium	Cd	112.40	Phosphorus	P	31.04
Caesium	Cs	132.81	Platinum	Pt	195.2
Calcium	Ca	40.07	Potassium	K	39.10
Carbon	C	12.00	Praseodymium	Pr	140.9
Cerium	Ce	140.25	Radium	Ra	226.0
Chlorine	Cl	35.46	Rhodium	Rh	102.9
Chromium	Cr	52.0	Rubidium	Rb	85.45
Cobalt	Co	58.97	Ruthenium	Ru	101.7
Columbium	Cb	93.5	Samarium	Sa	150.4
Copper	Cu	63.57	Scandium	Sc	44.1
Dysprosium	Dy	162.5	Selenium	Se	79.2
Erbium	Er	167.7	Silicon	Si	28.3
Europium	Eu	152.0	Silver	Ag	107.88
Fluorine	F	19.0	Sodium	Na	23.00
Gadolinium	Gd	157.3	Strontium	Sr	87.63
Gallium	Ga	69.9	Sulfur	S	32.06
Germanium	Ge	72.5	Tantalum	Ta	181.5
Glucinum [1]	Gl	9.1	Tellurium	Te	127.5
Gold	Au	197.2	Terbium	Tb	159.2
Helium	He	4.00	Thallium	Tl	204.0
Hydrogen	H	1.008	Thorium	Th	232.4
Indium	In	114.8	Thulium	Tm	168.5
Iodine	I	126.92	Tin	Sn	118.7
Iridium	Ir	193.1	Titanium	Ti	48.1
Iron	Fe	55.84	Tungsten	W	184.0
Krypton	Kr	82.92	Uranium	U	238.2
Lanthanum	La	139.0	Vanadium	V	51.0
Lead	Pb	207.20	Xenon	Xe	130.2
Lithium	Li	6.94			
Lutecium	Lu	175.0	Ytterbium (Neoytterbium)	Yb	173.5
Magnesium	Mg	24.32	Yttrium	Yt	88.7
Manganese	Mn	54.93	Zinc	Zn	65.37
Mercury	Hg	200.6	Zirconium	Zr	90.6

[1] Also called Beryllium, Be.

TABLE II. SPECIFIC GRAVITY AND PERCENTAGE OF ALCOHOL

(According to Squibb.)

Per cent alcohol by volume	Sp. Gr. at $\frac{25^\circ}{15.56}$ C.	Per cent alcohol by volume	Sp. Gr. at $\frac{25^\circ}{15.56}$ C.
1	0.9970	51	0.9246
2	.9953	52	.9226
3	.9938	53	.9205
4	.9922	54	.9184
5	.9909	55	.9164
6	.9893	56	.9143
7	.9876	57	.9122
8	.9868	58	.9100
9	.9855	59	.9081
10	.9846	60	.9056
11	.9831	61	.9034
12	.9816	62	.9011
13	.9801	63	.8989
14	.9793	64	.8969
15	.9787	65	.8947
16	.9773	66	.8923
17	.9759	67	.8895
18	.9746	68	.8870
19	.9733	69	.8846
20	.9726	70	.8821
21	.9719	71	.8796
22	.9706	72	.8771
23	.9692	73	.8746
24	.9678	74	.8719
25	.9668	75	.8689
26	.9655	76	.8665
27	.9646	77	.8641
28	.9631	78	.8616
29	.9617	79	.8583
30	.9603	80	.8558
31	.9594	81	.8530
32	.9582	82	.8500
33	.9567	83	.8476
34	.9556	84	.8444
35	.9538	85	.8414
36	.9521	86	.8384
37	.9507	87	.8352
38	.9489	88	.8326
39	.9473	89	.8291
40	.9456	90	.8258
41	.9438	91	.8223
42	.9424	92	.8191
43	.9402	93	.8156
44	.9382	94	.8118
45	.9363	95	.8083
46	.9343	96	.8044
47	.9323	97	.8003
48	.9307	98	.7960
49	.9288	99	.7914
50	.9267	100	.7865

TABLE III. TABLE OF FREEZING MIXTURES OF POWDERED ICE AND VARIOUS SALTS

100 parts of powdered ice at —1° mixed with the indicated weight of the following substances will give the temperature shown in the third column.

Substance	Parts by weight	Temperature deg.
Potassium sulphate	10	−1.9
Sodium carbonate crystals	20	−2.0
Potassium nitrate	13	−2.85
Potassium chloride	30	−10.9
Ammonium chloride	25	−15.4
Ammonium nitrate	45	−16.75
Sodium nitrate	50	−21.3
Sodium chloride	30	−22.4
Calcium chloride crystals ($CaCl_2+2H_2O$)	143	−50.

APPENDIX B. REAGENTS AND SOLUTIONS.

ACIDS

	100 c.c. contains
Acetic acid, glacial, sp. gr. 1.06	111.1 gms. CH_3COOH
Acetic acid, dilute, 1/2 normal (27 c.c. glacial acetic acid made up to 1 liter.)	3.0 gms. CH_3COOH
Hydrochloric acid, conc., sp. gr. 1.19	36.6 gms. HCl
Hydrochloric acid, dilute, 2/1 normal (200 c.c. conc. acid made up to 1 liter.)	7.3 gms. HCl
Hydrochloric acid, dilute, 1/10 normal (10 c.c. of conc. HCl made up to 1 liter.)	0.36 gm. HCl
Nitric acid, conc., sp. gr., 1.42	99.1 gms. HNO_3
Nitric acid, dilute, 2/1 normal (125 c.c. conc. acid made up to 1 liter.)	12.6 gms. HNO_3
Sulphuric acid, conc., sp. gr. 1.84	175.9 gms. H_2SO_4
Sulphuric acid, dilute, 2/1 normal (56 c.c conc. acid made up to 1 liter.)	9.8 gms. H_2SO_4
Sulphuric acid, dilute, 1/10 normal (2.8 c.c. conc. acid made up to 1 liter.)	0.5 gm. H_2SO_4

ALKALIES

	100 c.c. contains
Ammonia, conc., sp. gr. 0.88	31.0 gms. NH_3
Ammonia, dilute, 2/1 normal (105 c.c. conc. ammonia made up to 1 liter.)	3.4 gms. HN_3
Barium hydroxide, 1/4 normal (40 gms. $Ba(OH)_2-8H_2O$ dissolved in water and made up to 1 liter.)	4.2 gms. $Ba(OH)_2$
Sodium hydroxide, sp. gr. 1.34 (410 gms. 98% caustic soda dissolved in water and made up to 1 liter.)	40.0 gms. $NaOH$
Sodium hydroxide, dilute, 2/1 normal (82 gms. 98% caustic soda dissolved in water and made up to 1 liter.)	8.0 gms. $NaOH$
Sodium hydroxide, dilute, 1/10 normal (4.1 gms. 98% caustic soda dissolved in water and made up to 1 liter.)	0.4 gm. $NaOH$

SALT SOLUTIONS

Ammonium chloride, 2/1 normal (107 gms. NH_4Cl dissolved in water and made up to 1 liter.)	10.7 gms.
Ammonium carbonate, 1/1 normal (48 gms. $(HN_4)_2CO_3$ dissolved in water and made to 1 liter.)	4.8 gms.

	100 c.c. contains
Ammonium molybdate (124 gms. $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ dissolved in cold water and made up to 1 liter.)	11.5 gms.
Barium chloride, 1/1 normal (122 gms. $BaCl_2\cdot 2H_2O$ dissolved in water and made up to 1 liter.)	10.4 gms.
Calcium chloride, 1/1 normal (110 gms. $CaCl_2\cdot 6H_2O$ dissolved in water and made up to 1 liter.)	5.5 gms.
Copper sulphate (Fehling's solution A, about one-half normal (69.28 gms. $CuSO_4\cdot 5H_2O$ dissolved in water and made up to 1 liter.)	4.0 gms.
Ferric chloride, 1/2 normal (27 gms. dissolved in water and made up to 1 liter.)	2.7 gms.
Iodine solution, 1/10 normal (13 gms. of iodine are dissolved in a solution of 30 gms. KI in 250 c.c. of water and made up to 1 liter.)	1.3 gms.
Lead acetate, 1/2 normal (95 gms. $Pb(C_2H_3O_2)_2\cdot 3H_2O$ dissolved in water and made up to 1 liter.)	8.1 gms.
Lead acetate, basic, 1/2 normal (56 gms. litharge dissolved in a solution of 95 gms. lead acetate in about 800 c.c. water and made up to 1 liter.)	13.6 gms.
Potassium bichromate, 1/2 normal (24.55 gms. $K_2Cr_2O_7$ dissolved in water and made up to 1 liter.)	2.4 gms.
Potassium forrocyanide, 1/2 normal (5.3 gms. $K_4Fe(CN)_6\cdot 3H_2O$ dissolved in water and made up to 1 liter.)	4.6 gms.
Potassium ferricyanide, 2/1 normal (44 gms. $K_3Fe(CN)_6$ dissolved in water and made up to 1 liter.)	4.4 gms.
Potassium permanganate, 1/20 normal (1.58 gms. $KMnO_4$ dissolved in water and made up to 1 liter.)	0.16 gm.
Mercuric chloride, 1/2 normal (68 gms. $HgCl_2$ dissolved in water and made up to 1 liter.)	6.8 gms.
Sodium carbonate, 1/10 normal (14.3 gms. $NaCO_3\cdot 10H_2O$ dissolved in water and made up to 1 liter.)	0.5 gm.
Sodium chloride, 2/1 normal (117 gms. NaCl dissolved in water and made up to 1 liter.)	11.7 gms.
Silver nitrate, 1/10 normal (17 gms. $AgNO_3$ dissolved in water and made up to 1 liter.)	1.7 gms.
Sodium nitrite, 1/10 normal (6.9 gms. $NaNO_2$ dissolved in water and made up to 1 liter.)	0.7 gm.

	100 c.c. contains
Sodium phosphate, 1/1 normal.................... (119.5 gms. $Na_2HPO_4 \cdot 12H_2O$ dissolved in water and made up to 1 liter.)	4.7 gms.
Uranium acetate, 1/10 normal.................... (21.3 gms. $UrO_2(C_2H_3O_2)_2 \cdot 2H_2O$ dissolved in water and made up to 1 liter). (1 c.c.= 0.00355 gm. P_2O_5).	2.1 gms.

SPECIAL REAGENTS

Acid sodium acetate solution.

100 gms. sodium acetate
30 c.c. glacial acetic acid } are dissolved in water and made up to 1 liter.

Alcoholic caustic soda.

20 gms. sodium or 20 gms. caustic soda. } are dissolved in alcohol and made up to 1 liter.

Ammonium sulphate. (Saturated solution.)

780 gms. ammonium sulphate are dissolved in water and made up to 1 liter.

Barfoed's reagent.

66 gms. copper acetate, 10 c.c. glacial acetic acid are dissolved in water and made up to 1 liter.

Benedict's qualitative reagent for glucose, etc.

173 gms. sodium citrate
100 gms. anhydrous sodium carbonate } are dissolved in about 600 c.c water and filtered into a 1-liter measuring cylinder and diluted to about 850 c.c.

17.3 gms. $CuSO_4 \cdot 5H_2O$ are dissolved in 100 c.c. water and diluted to 150 c.c. This solution is added with constant stirring to the citrate carbonate solution. The mixture is immediately ready for use.

Bial's pentose reagent.

1 gram orcinol, dissolved in 500 c.c. 30% HCl, to which 30 drops of 10% ferric chloride have been added.

Bromine water. 25 c.c. of bromine in 1 liter of water.

Fehling's solution. Equal volumes of *A* and *B*.

A. 69.28 gms. of copper sulphate dissolved in water and made up to 1 liter.

B. 346 gms. of Rochelle salt (NaK tartrate), and 260 gms. of NaOH dissolved in water and made up to 1 liter.

Formalin. Commerical 40% solution of formaldehyde.

Gies' biuret reagent. This reagent consists of 2/1 normal NaOH solution to which enough 1/10 normal copper sulphate has been added to impart a slight though distinct blue color to the clear liquid. The copper sulphate should be added, drop by drop, with constant stirring.

Glyoxylic acid solution. Benedict's modification of Hopkins-Cole Reagent. Ten gms. of powdered magnesium are mixed with enough water to liberally cover the magnesium. 250 c.c. of saturated oxalic acid is now added slowly, and the solution kept cool, by allowing running water to flow over the flask during the addition of the acid. The magnesium oxalate is filtered off. The filtrate is acidified with acetic acid to prevent the partial precipitation of magnesium on long standing, and made up to 1 liter with water.

Magnesia mixture.
55 gms. magnesium chloride
70 gms. ammonium chloride
125 c.c. of NH_4OH (sp. gr. .880)
are dissolved in water and made up to 1 liter.

Magnesium sulphate (saturated solution).
600 gms. of cryst. $MgSO_4$ are dissolved in water and made up to 1 liter.

Millon's reagent.
400 gms. mercury (30 c.c.) are dissolved in 570 c.c. of conc. nitric acid. The solution is then diluted with two volumes of water.

Mörner's reagent.
Thoroughly mix 1 volume of formalin, 45 volumes of distilled water, and 55 volumes of conc. H_2SO_4.

Molisch's reagent.
144 gms. of alpha-naphthol are dissolved in alcohol and made up to 1 liter with alcohol.

Nylander's solution.
40 gms. Rochelle salt
20 gms. bismuth subnitrate
are dissolved in 1 liter of 2/1 NaOH.

Obermayer's reagent.
3 gms. of ferric chloride are dissolved in 1 liter of conc. hydrochloric acid.

Oxalic acid (saturated solution).
100 gms. of oxalic acid are dissolved in 1 liter of water.

Pavy's solution.
120 c.c. Fehling's solution
300 c.c. NH_4OH (sp. gr. .880)
are made up to 1 liter with water.

Phenol solution.
20 gms. phenol are diluted to 1 liter with water.

Phosphotungstic acid solution.
50 gms. of phosphotungstic acid and 30 c.c. of conc. H_2SO_4 are dissolved in water and made up to 1 liter.

Picric acid (saturated solution).
12 gms. of picric acid are dissolved in water and made up to 1 liter.

Sodium bisulphite (saturated solution).
600 gms. of sodium bisulphite are dissolved in water and made up to 1 liter.

Sodium chloride (saturated solution). 370 gms. of NaCl are dissolved in water and made up to 1 liter.

Schweitzer's reagent.
10 gms. of ammonium chloride are dissolved in 250 c.c. of 1/2 normal copper sulphate, and NaOH solution is added until precipitation is complete. The mixture is filtered and the pptd. cupric hydroxide is washed with water and then dissolved in 1 liter of conc. ammonium hydroxide.

Tannic acid solution.

100 gms. tannic acid
25 gms. sodium acetate
25 gms. sodium chloride
50 gms. glacial acetic acid
are dissolved in water and made up to 1 liter.

Tartaric acid (saturated solution).

750 gms. of tartaric acid are dissolved in water and made up to 1 liter.

Trichloracetic acid solution.

100 gms. of trichloracetic acid are dissolved in water and made up to 1 liter.

Uffelmann's reagent.

Add 1/2 normal solution of ferric chloride to a 2% aqueous solution of phenol until an amethyst-blue is obtained.

INDICATORS

Alizarin red.

10 gms. sodium alizarin sulphonate are dissolved in water and made up to 1 liter.

Cochineal tincture.

5 gms. cochineal are extracted with 150 c.c. alcohol + 100 c.c. water for several days; the solution is then filtered.

Congo red.

1 gm. Congo red is dissolved in water and made up to 1 liter.

Litmus.

10 gms. litmus are finely powdered and extracted with 50 c.c. of hot water The blue liquid is decanted and made up to 1 liter.

Methyl orange.

1 gram methyl orange is dissolved in 500 c.c. alcohol and made up to 1 liter with water.

Methyl violet.

1 gram methyl violet is dissolved in water and made up to 1 liter.

Phenolphthalein.

10 gms. phenolphthalein are dissolved in alcohol and made up to 1 liter with alcohol.

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